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IMPACT OF CLIMATE VARIABILITY ON THE FREQUENCY AND SEVERITY OF  
ECOLOGICAL DISTURBANCES IN GREAT BASIN BRISTLECONE PINE SKY  
ISLAND ECOSYSTEMS

by

Curtis A. Gray

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Ecology

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Logan, UT

2017

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## ABSTRACT

Impact of Climate Variability on the Frequency and Severity of Ecological Disturbances  
in Great Basin Bristlecone Pine Sky Island Ecosystems

by

Curtis A. Gray, Doctor of Philosophy

Utah State University, 2017

Major Professor: Dr. Michael Jenkins  
Department: Wildland Resources

Great Basin bristlecone pine (GBBP) (*Pinus longaeva* Bailey) is one of the longest-lived organisms on Earth, and is one of the most highly fragmented high elevation conifer species. Throughout the Great Basin of the Intermountain West, GBBP are being impacted by changing disturbance regimes, invasive species, and climate change. To better understand the effects of climate variability and ecological disturbances in GBBP systems, three studies were designed and implemented. The first characterized the distribution of forest fuel in stands of GBBP and predicted how fuels may change under future climate scenarios. Using the Forest Inventory Analysis (FIA) plot variables of tree species, height, diameter at breast height (DBH), canopy base height (CBH), coarse (CWD) and fine (FWD) woody debris across elevational gradients, this study examined the effects of changes to fuel loading on predicted changes in fire behavior and severity. All classes of FWD decreased with elevation, and only 1000-hr fuels remained constant across elevational transects. This, combined with lower CBH and foliar moisture

and increasing temperatures due to climate change, suggested increased fire potential at the GBBP treeline. The second study examined the role of volatile organic compounds (VOCs) and tree chemistry and their response to the environment. VOCs and within needle chemistry were collected and analyzed along elevational gradients near the northern and southern limits of GBBP. Random Forest analysis distinguished elevation using VOCs, with 83% accuracy, and identified the compounds most important for classification. Ordination revealed that temperature, heat load index, and relative humidity were each significantly correlated with VOCs. Within-needle chemistry provided less predictive value in classifying elevation (68% accuracy) and was correlated only with heat load index. These findings suggest that GBBP VOCs are highly sensitive to the environment. The final study explored the role of VOCs in host selection of mountain pine beetle (MPB). Mountain pine beetles oriented toward VOCs from host limber pine (*Pinus flexilis* James) and away from VOCs of non-host GBBP using a Y-tube olfactometer. When presented with VOCs of both trees, females overwhelmingly chose limber pine over GBBP. While there were only a few notable differences in VOCs collected from co-occurring GBBP and limber pine, 3-carene and D-limonene were produced in greater amounts by limber pine. There was no evidence that 3-carene is important for beetles when selecting trees, however, addition of D-limonene to GBBP VOCs disrupted the ability of beetles to distinguish between tree species. Climate change will impact how forests are managed and this research could provide insight into the mechanisms underlying the incredible longevity of this iconic tree species.

## PUBLIC ABSTRACT

Impact of Climate Variability on the Frequency and Severity of Ecological Disturbances  
in Great Basin Bristlecone Pine Sky Island Ecosystems

Curtis A. Gray

Many high elevation conifer species, including high elevation five needle pines, are declining throughout western North America. Warming temperatures, mountain pine beetle, white pine blister rust and alteration of naturally occurring fire regimes represent an interactive set of circumstances leading to greater risk. The loss of these treeline pines can detrimentally impact biodiversity and valuable ecosystem services including wildlife habitat, watershed and soil protection, aesthetics and recreation. Great Basin bristlecone pine ecosystems are naturally highly fragmented because of their elevational requirements. However, they may become even more fragmented due to combined impacts of warming temperature, insects and diseases listed above. This study increased the knowledge of Great Basin bristlecone pine ecology by examining response to climate change with respect to fire, fuels and tree chemistry. The first study examined alteration of the fire regime and showed that fuels in Great Basin bristlecone pine decreased with elevation. Yet, canopy fuels that are more susceptible to fire, suggested fire potential may increase at higher elevations with warming air temperatures, which could threaten the oldest individuals of this iconic species. Examination of tree chemistry to environmental gradients (like elevation and temperature) demonstrated a clear response to climate induced environmental stress. This has the potential to alter flammability and the effectiveness of tree defenses to mountain pine beetle. Lastly, this research determined

that volatile organic compounds emitted from Great Basin bristlecone pine foliage influence host selection for mountain pine beetle. All three of these studies will aid in developing unique forest management practices to increase forest resilience of treeline species and could provide insight into the mechanisms underlying the incredible longevity of Great Basin bristlecone pine.

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Curtis Gray



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## CHAPTER 1

### INTRODUCTION

Many high elevation conifer species, including all high elevation five needle pines, are declining throughout western North America (Gibson et al., 2008). Warming temperatures, mountain pine beetle, white pine blister rust and alteration of naturally occurring fire regimes (Gibson et al., 2008) interact and lead to decline. High elevation pine forests provide important ecosystem services (Schoettle, 2004), including stabilizing soil, improving snow retention, pioneering regeneration of alpine sites after fire, providing habitat for wildlife, and facilitating growth of other tree species (Baumeister and Callaway, 2006). Great Basin bristlecone pine (GBBP) (*Pinus longaeva* Bailey) is one of the longest-lived, non-clonal organisms on Earth, and is one of the most highly fragmented high elevation conifer species (IUCN, 2003). This species has a wide geographic distribution, but limited elevation range (between approximately 2100 and 3500m), and occurs in fragmented groves throughout the Great Basin. The highest elevation stands often occur in climax forests that form ecological “sky islands”. Depending upon elevation zone, the pine also grows as a minor to major seral in mixed conifer forests.

Sky islands are mountains that are isolated by surrounding lowlands of a dramatically different ecosystem, which, in combination with altitudinal zonation, has significant implications for natural habitats. Endemism, vertical migration, and relict populations are some of the phenomena unique to sky islands (MacArthur and Wilson, 1967). While sky island theory is an offshoot of island biogeography, developed to explain species richness of actual islands (Brown, 1978), sky island theory has since been



extended to mountains, lakes, fragmented forest, and even natural habitats surrounded by human-altered landscapes and now reference any ecosystem isolated by unlike ecosystems. This is the case in the high isolated peaks of the Great Basin, in which fragmented, isolated populations of GBBP may develop unique evolutionary traits. GBBP occurring in sky islands are abundant on the landscape where present, but rare in total distribution, and face risks from demographic variability, fragmentation, loss of fitness, and loss of evolutionary responsiveness as the population becomes rarer (Courchamp et al., 2008). Although GBBP ecosystems are naturally highly fragmented because of their elevation requirements, they may become even more so due to combined threats of warming temperatures, introduced species, insects and diseases.

Climate change is predicted to significantly alter the frequency and severity of disturbances, such as fire, that shape forest ecosystems (Logan and Bentz, 1999). Climate change impacts may be especially acute in sky islands of the Great Basin as warming temperatures drive montane and alpine ecosystems upslope, resulting in overstory tree mortality at the lower margins of distribution. Minor seral stands are much more susceptible to changing climate regimes and increased ecological disturbances such as fire because they occur at lower elevations and in proximity to higher fire frequency vegetation communities (Westerling et al., 2006).

Little previous research has been done on describing the distribution of GBBP pine forests, fuel complexes, foliar terpene chemistry or the effect of the disturbance regime on GBBP regeneration. The objectives of this research are to:

1. Develop a distribution model of GBBP forests in Great Basin sky islands,
2. Characterize the distribution of forest fuel under individual trees and in stands of GBBP and predict how fuels may change under likely future climate scenarios,
3. Describe the volatile terpene foliar chemistry of GBBP and evaluate changes in terpene chemistry across environmental gradients and under various climate change scenarios and,
4. Use volatile terpene foliar chemistry to better understand host selection of mountain pine beetle (MPB), elucidating how GBBP escapes attack by MPB and provide insight into mechanisms underlying the longevity of this tree species

The work proposed here will examine the current distribution of GBBP, potential alteration of fire regime and regeneration dynamics from climate warming, terpenes as a possible defense mechanism against MPB, flammability, an indicator of stress, and project how combined these factors will affect pine distribution and resiliency into the future. By increasing our understanding of both basic and applied forest dynamics, managers in the Intermountain West will be better equipped to implement sound forest management practices to increase forest resilience with future climate uncertainty.

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## CHAPTER 2

### USING MULTIVARIATE METHODS TO PREDICT THE DISTRIBUTION OF GREAT BASIN BRISTLECONE PINE FORESTS

#### **Abstract**

Accurate maps are necessary to make informed decisions on ecology species and habitat. This is especially true with a fragmented species such as Great Basin bristlecone pine (GBBP), which occurs on ‘islands’ of high elevation in the Basin and Range peaks of California, Nevada, and Utah. Due to the inaccessibility of many of the sites that this species occurs, information on their location and abundance is incomplete, and thus is needed. Understanding the distribution of this species is required to evaluate their potential response to disturbances such as fire and climate change. I modeled the distribution of GBBP using widely available topographic and spectral variables calculated from a geographic information system (GIS). I tested several multivariate statistical models to produce a GIS layer (map) that provides a foundation to examine large scale changes to GBBP in later chapters.

#### **1. Introduction**

High elevation, five needle pines are rapidly declining throughout western North America due to warming temperatures, mountain pine beetle (*Dendroctonus ponderosae* Hopkins), white pine blister rust, introduction of non-native species, and alteration of the natural fire regime (Gibson et al., 2008). One five needle pine of special concern is the Great Basin bristlecone pine (*Pinus longaeva* Bailey). An icon of western forests, Great Basin bristlecone pines (GBBP) are the oldest non-clonal organism known, and their

rings are often used in dendrochronology studies as records of historical climate. GBBP are medium-sized trees, reaching an average of 5 to 15 m tall, and are found on the high mountain peaks of the southwestern United States (Moore et al., 2008). The highest elevation stands often occur in climax forests that form ecological “sky islands” which result in a highly fragmented distribution in which small populations exist as islands surrounded by dramatically different ecosystems, such as cropland, pasture, pavement, deserts, or even barren land. Depending upon elevation zone, the pine also grows as a minor to major seral in mixed conifer forests (Gray and Jenkins, 2017).

Sky islands are a concept from island biogeography, which examines the factors that affect the species diversity of isolated communities. Fluctuations in climate, precipitation or disturbance regime, which could be corrected for in large populations, can be catastrophic in small, isolated populations. Endemism, vertical migration, and relict populations are some of the phenomena unique to sky islands (Wilson and MacArthur, 1967). Thus fragmentation of habitat is an important cause of species extinction (Rosenzweig, 1995) Fragmentation metrics such as patch size, edge-to-edge distance, habitat configuration, or amount of edge are useful in assessing genetic risk to the over species population (Hargis et al., 1998).

A primary goal of my research is to improve understanding of future changes on GBBP distribution and resiliency as described in my research objectives in Chapter 1. A spatially-explicit distribution landscape model representing GBBP distribution across elevational and geographic gradients was developed to address the research question: What is the current spatial distribution of GBBP? Several mapping efforts have included GBBP, however all have fallen short in providing accurate locations of GBBP groves

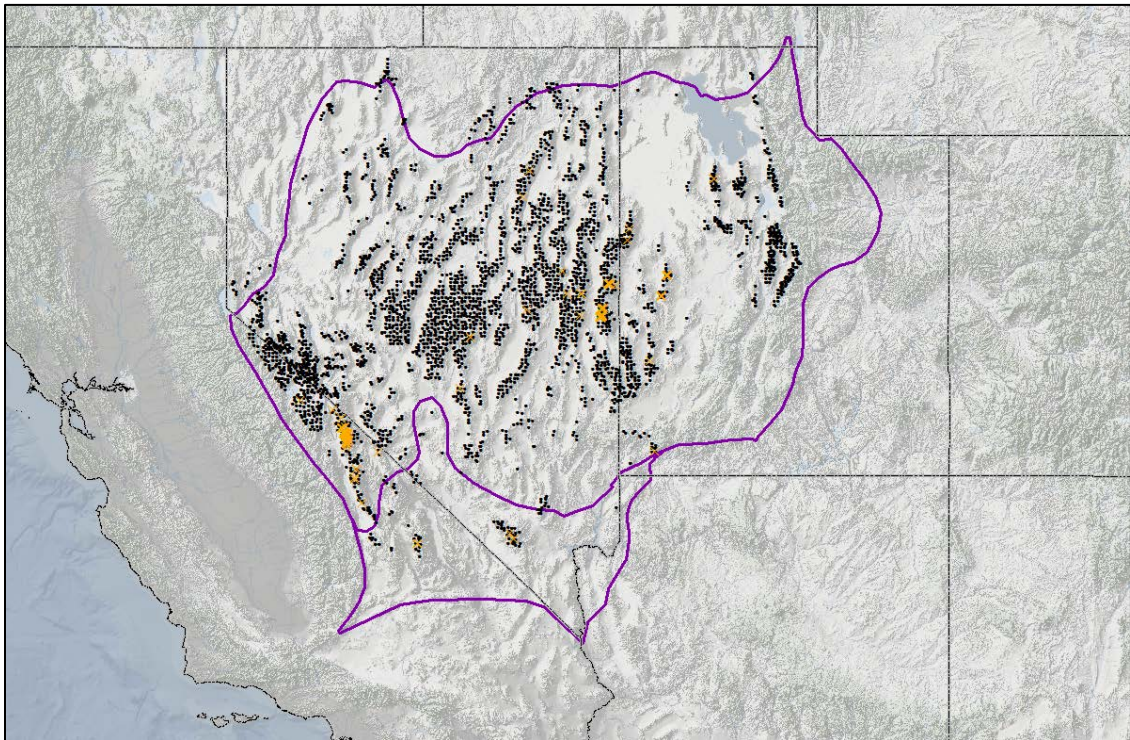
(Little and Viereck, 1971; Lowry et al., 2007; Rollins, 2009). For example, USFS species distribution maps show locations of GBBP, however the accuracy is poor and stands are often depicted in valley bottoms or as simplified large ellipses (Little and Viereck, 1971). The southwest GAP land cover layer (Lowry et al., 2007) and LANDFIRE (Rollins, 2009) have modeled the distribution of western vegetation types, but GBBP is lumped with limber pine (*Pinus flexilis* James), which is much more widely distributed than GBBP. This species distribution model will be the foundation to examine large-scale changes to GBBP in later chapters.

## **2. Methods**

### *2.1. Study Area*

The study area for this project is the Great Basin mountains in North America, which includes parts of California, Nevada, Utah, and Arizona (Fig. 1). The Great Basin is the largest area of contiguous endorheic watersheds, or closed drainage basins, in North America. It is noted for both arid conditions and basin and range topography (Edwards, 1976). Although mostly within the North American Desert ecoregion, portions of the Great Basin extend into the forested mountain and Mediterranean California ecoregions. The semi-arid areas of the forested mountain ecoregion include the White Mountains and Inyo Mountains. (NPS, 2010). Takhtajan (1986) defined the Great Basin Floristic Province to extend well beyond the boundaries of the hydrographically defined Great Basin to include the Snake River Plain, the Colorado Plateau, the Uinta Basin, and parts of Arizona north of the Mogollon Rim. Additional stands of GBBP were sampled and modeled in the Southern Great Basin (southern polygon Fig. 1), and the Colorado

Plateau. Climate varies throughout the Great Basin by elevation and latitude. Higher elevations tend to be cooler and receive more precipitation. The western areas of the basin tend to be drier than the eastern areas because of the rain shadow of the Sierra Nevada Mountains. Most of the basin experiences a semi-arid or arid climate with warm summers and cold winters (NPS, 2010).



**Fig. 1.** The Great Basin Floristic Province (*northern purple polygon*) and GBFP data points. The black dots are the absent points (*all from FIA*), and the orange X's are the present points. The southern purple polygon (*Mojave Desert*) contains two stands of GBFP outside of the Great Basin Floristic Province and GBFP. Additional stands were modeled to the east on the Colorado Plateau.

## *2.2. Field Data*

The data used to calibrate the model (termed the training dataset) is a compilation of field-visited sites (Gray, unpublished data 2012), herbarium records (Global Biodiversity Information Facility - [www.gbif.org](http://www.gbif.org)); and Forest Inventory and Analysis data – FIA - <https://www.fia.fs.fed.us>) which portray GBBP presence/absence. All sampled areas are groves of GBBP (Fig. 1). Many of the points collected during the summer of 2012 are located in Great Basin National Park, the Mt. Moriah Wilderness, and portions of the Humboldt-Toiyabe National Forest, which lie approximately 290 miles (470 km) north of Las Vegas and are made up of a combination of federally protected (US Forest Service and National Park Service) wilderness areas. Absence points were all obtained from the FIA Program, which is a national program that gathers annual inventory data on a 4.8 km grid across forested areas. All forested FIA points within the study area boundary that were above 2000 m in elevation, but that did not contain GBBP, were utilized as absent locations. The resulting final dataset, all with X/Y spatial coordinates, contains 496 GBBP presence locations, and 3399 locations in which GBBP was absent.

## *2.3. Independent Variables*

I selected 12 independent variables to represent abiotic (topographic and climate) factors and describe the ecological niche of GBBP. The variables are elevation, slope, aspect, blue, green, red and infra-red radiation, NDVI, brightness, greenness, wetness, and texture. The terrain variables were derived from USGS 10 meter digital elevation models and the spectral variables were derived from Landsat images (30 m spatial



resolution) collected in summer 2012. All spectral variables were resampled to 10 m to match the topographic variables.

#### *2.4. Spectral Variables*

The predictive model used several spectral variables, all derived from a mosaic of 2012 Landsat 5 TM images. Band 1, the blue band, 0.45-0.52  $\mu\text{m}$ , distinguishes soil from vegetation and deciduous from coniferous vegetation. Band 2, green, 0.52-0.60  $\mu\text{m}$ , emphasizes peak vegetation, which is useful for assessing plant vigor. Band 3, red, 0.63-0.69  $\mu\text{m}$ , identifies vegetation. Band 4, near infrared (NIR), 0.77-0.90  $\mu\text{m}$ , emphasizes biomass content. Additionally, texture of Band 3 was calculated. Texture is the frequency of tonal change on the photographic image. Texture, a product of shape, size, pattern, shadow, and tone, is produced by aggregating unit features that may be too small to be discerned individually on an image. Interpretation can often distinguish between features with similar reflectance based on texture (Lillesand and Kiefer, 1979). As an example, smooth green grass has a smooth texture, while tree crowns have a course texture. One would expect bristlecone pine to have a course texture, due to the sparse nature of the stands, combined with high soil reflectance in the background. Thus, texture is helpful for identifying GBBP on Landsat images.

A special principal components transformation (Table 1), called the Tasseled Cap transformation, was applied to 6 Landsat TM spectral bands, creating three more variables – brightness, greenness and wetness. This transformation is a special case of principal components analysis, which transforms the image data to a new coordinate system with a new set of orthogonal axes. The primary axis, brightness, is statistically

derived and is calculated as the weighted sum of reflectance of all spectral bands. Orthogonal to the first component, the second component, greenness, shows variations in the vigor of green vegetation. Wetness is orthogonal to the first two components and represents soil moisture and water (Kauth and Thomas, 1976). The Tasseled Cap transformation provides an analytical way to detect and compare changes in vegetation, soil, and man-made features over short- and long-term time periods.

**Table 1**

Coefficients for the tassle cap functions for Landsat TM bands 1-5 and 7 (Crist and Cicone, 1984)

TM Band	1	2	3	4	5	7
Brightness	0.3037	0.2793	0.4743	0.5585	0.5082	0.1863
Greenness	0.2848	-0.2435	-0.5436	0.7243	0.084	-0.1800
Wetness	0.1509	0.1973	0.3279	0.3406	-0.7112	-0.4572

The Normalized Difference Vegetation Index (NDVI) assesses post-disturbance vegetation recovery between a disturbance event and field sampling. It was calculated for each image. NDVI, an indicator of live green vegetation, is sensitive to photosynthetically active biomass (Tucker, 1979) and is correlated with leaf area index (Asrar et al., 1984).

### 2.5. Topographic Variables

Topographic variables used in the predictive model were: elevation (meters above sea level), slope - gradient as a steepness measure of the maximum rate of elevation change, indicated as a percentage of angle (Burrough et al., 2015), and aspect as an indication of which way the slope is facing defined as the compass direction of the gradient (Burrough et al., 2015). Other topographic variables like curvature (whether a

surface is concave or convex), were examined in preliminary models but were not found to be important in predicting GBBP in the final model.

### **3. Statistical Analyses**

#### *3.1. Statistical Classification*

The general goal of classification statistical models is to identify good predictors (variable X) for describing if a species of interest occupies a particular ecological niche, which is the response variable (Y) of any sample of the same distribution. To describe the fundamental or potential niche of a species, we want to know the abiotic and biotic factors that limit the species, for example, the thermal, moisture and light regimes that determine species range limits at larger spatial scales or the nutrient requirement of the soils. We must assume that species distributions are in equilibrium with the climate (Lenihan, 1993). To describe these factors we use environmental surrogates such as terrain variables. Elevation can substitute for temperature and precipitation, slope and aspect can be a surrogate for radiation regime and moisture availability, and landform, hillslope position, and catchment position for soil moisture, erosion, and deposition (Franklin, 1995).

Linear discriminant analysis (LDA), generalized linear models (GLMs), generalized additive models (GAMs), classification and regression trees (CART), and random forests (RF) are all statistical methods that classify, or separate, observations into two or more classes of objects or events. The goal of LDA is to classify observations into *a priori* or known groups. In the instance of species distribution modeling (SDM), these classes are the binary response of the species of interest being present or absent. A LDA

of the training data is implemented to form a set of linear functions (equations) or estimated coefficients that express the degree of support for each class. The assigned class for each  $y$  (in our case presence or absence) is the class that receives the highest support after evaluation of all functions (Lattin et al., 2003). While LDA strength is its simplicity of interpretation and acceptance, LDA is most useful when population distributions are known. The Coefficients of the Linear Discriminant Function is similar to regression and has the form:  $C_k = C_{k0} + C_{k1}X_1 + \dots + C_{km}X_m$ ;  $C_k$  is the classification score for the  $k^{th}$  group. For each observation, we compute the classification score with the coefficients according to the equation and assign the observation to the group with the highest score. The coefficients are helpful in deciding which variables have more weight in classification, the higher coefficient means the variable is a better classifier for that group. Ecological data often violate the assumptions of the linear model discussed above. Generalized linear models (GLM) are extensions of linear models that can cope with non-normal distributions of the response variable using a link function (Agresti, 1996). Some strengths of GLM are the flexibility to transform predictor variables that follow a curvilinear response (e.g., species count data), and ability to handle categorical predictor variables (e.g., dolomite soil, land cover) which is done by coding them as dummy variables. GLM are well suited to examine simple relationships between an individual environmental variable and species data, however the number of candidate predictor variables is frequently large in ecology and this makes a thoughtful approach to model selection particularly important. GLM must follow the assumptions of logistic regression, which are conditional probabilities are a logistic function of the predictor variables, no

important variables are omitted, no extraneous variables are included, and observations are independent.

Generalized additive models (GAM) are a flexible and automated approach to identifying and describing non-linear relationships. GAM make another generalization to GLM to incorporate nonlinear forms of predictors and relate them to the response variable. In GAM, the observed values  $Y$  are assumed to be of some exponential family distribution, and  $\mu$  is still related to the model predictors via a link function. Like GLM, GAM assume that  $Y$  are independent and have a specified distribution (for example normal, binomial or Poisson distributions). The key difference is that coefficients of the GLM are replaced by some smoothing function of at least some (possibly all) covariates (Wood, 2017). Gaining understanding of a species ecological niche is difficult with GAM because they cannot calculate species response parameters such as optimum habitat and tolerance thresholds. Another limitation of GAM, either for exploration or prediction, is that they are additive, and it is difficult to introduce interaction terms. If interaction terms are necessary it might be better to use decision trees (discussed below) which are particularly good for identifying interactions among predictor variables (Franklin, 2010).

Classification and Regression Trees (CART) are collectively known as tree-based methods, when used with a categorical response. CART uses recursive partitioning, in which the decision tree model is to partition the data into subgroups where the response variables have similar values or are members of the same class. Each of the terminal nodes of the tree represents a cell of the partition, and includes a simple model which applies in that cell only (Breiman et al., 1984). If you continue to partition the data until every observation is classified, your result is large trees than tend to overclassify the data.

To avoid this, partitioning is usually stopped when the resulting split does not achieve some defined level of increased homogeneity (or explained deviance), or when the resulting subsets would have less than some minimum number of members. Tree-based methods characterize interactions between variables extremely well (Breiman et al., 1984), characterizing threshold effects of predictor variables in an often simpler way than linear or smoothing responses. However, while CART is good at handling large datasets, trees require large samples to detect patterns.

Computationally intensive methods have been developed that address some of the shortcomings of CART. These methods are known as ensemble models because they involve estimating a large number of tree models based on subsets of the data and then averaging the results. RF is an ensemble model that builds a large number of trees and averages the prediction (Breiman, 2001). In order to avoid developing a tree model that is not over fit to data, a method known as “bagging” is used by repeatedly sampling the data with replacement and developing trees for each dataset using some stopping rule. The “out-of-bag” (test) sample, the set of observations held back, is used to estimate model error and variable selection or importance. (Breiman, 2001). For each decision tree there is a misclassification error rate calculated from the out-of-bag sample. The difference between this error rate and the error rate calculated by randomly assigning the values of a predictor variable, and then passing the test data down the tree to get new predictions, is a measure of the importance of that predictor (Cutler et al., 2007). Partial dependence plots of these splits determine the most important variables (Cutler et al., 2007). The tendency to over-fit the data is overcome by averaging the predictions from a large (500 – 2000) number of models based on subsets of the data. RF have higher prediction accuracy than

ordinary decision trees (Cutler et al., 2007). The statistical classifiers that were fit to the training data set were Linear Discriminant Analysis, Classification Trees, Random Forest, and two other ensemble model, Support Vector Machines, and Gradient Boosting Machines.

#### **4. Results**

I tested five multivariate models to classify and predict presence/absence of GBBP. The RF model provided the best fit (Table 2). It classified the training sites correctly 98.2% of the time and was chosen to select the most important variables to predict the presence of GBBP. Visual inspection of the resulting map showed this model better predicted GBBP groves in sites that were field verified than the previous mapping efforts mentioned above (Little and Viereck, 1971; Lowry et al., 2007; Rollins, 2009). More field validation is necessary to further develop this model for use in management decisions. While the classification tree (Table 2) performed slightly worse than RF, it is interesting to look at the interpretation. The first node split on elevation (elevations < 3009) with all observations < 3009 meters classified as absent of GBBP. The second node (NDVI < 97.5) classified GBBP present only in samples with an NDVI value greater than 97.5. This is plausible, as GBBP is limited to specific elevation ranges. NDVI (along with NIR) is useful to distinguish between barren alpine areas and forested stands.

**Table 2**

Accuracy measurements for predictions of presence of GBBP in the Great Basin Floristic Region. ( $N = 3895$ )

**Random Forest (all variables), Data=BCP,**  
out-of-bag accuracies

Actual Class	Predicted		Total	class.error
	Absent	Present		
Absent,0	3369	30	3399	0.00883
Present,1	39	457	496	0.07863
Total	3408	487	3895	

PCC = 98.23 %  
 Specificity = 99.12 %  
 Sensitivity = 92.14 %  
 k = 0.92  
 AUC = 0.995

Includes:  
 Northing  
 Easting  
 Proximity

**Random Forest (9 variables), Data=BCP,**  
out-of-bag accuracies

Actual Class	Predicted		Total	class.error
	Absent	Present		
Absent,0	3353	46	3399	0.01353
Present,1	49	447	496	0.09879
Total	3402	493	3895	

PCC = 97.59 %  
 Specificity = 98.65 %  
 Sensitivity = 90.32 %  
 k = 0.891  
 AUC = 0.994

**Classification Tree, Data=BCP,**  
crossvalidation accuracies

Actual Class	Predicted		Total
	Absent	Present	
Absent,0	3337	62	3399
Present,1	74	422	496
Total	3411	484	3895

PCC = 96.51 %  
 Specificity = 98.18 %  
 Sensitivity = 85.08 %  
 k = 0.841  
 AUC = 0.958

**Support Vector Machines, Data=BCP,**  
resubstitution accuracies

Actual Class	Predicted		Total
	Absent	Present	
Absent,0	3356	43	3399
Present,1	52	444	496
Total	3408	487	3895

PCC = 97.56 %  
 Specificity = 98.76 %  
 Sensitivity = 89.31 %  
 k = 0.704  
 AUC = 0.922

**Gradient Boosting Machines, Data=BCP,**  
resubstitution accuracies

Actual Class	Predicted		Total
	Absent	Present	
Absent,0	3331	68	3399
Present,1	64	432	496
Total	3395	500	3895

PCC = 96.61 %  
 Specificity = 98 %  
 Sensitivity = 87.1 %  
 k = 0.848  
 AUC = 0.988

**Linear Discriminant Analysis, Data=BCP,**  
resubstitution accuracies

Actual Class	Predicted		Total
	Absent	Present	
Absent,0	3252	147	3399
Present,1	43	453	496
Total	3295	600	3895

PCC = 95.12 %  
 Specificity = 95.68 %  
 Sensitivity = 91.33 %  
 k = 0.799  
 AUC = 0.98

**Random Forest, Data=BCP,**  
out-of-bag accuracies

Actual Class	Predicted		Total	class.error
	Absent	Present		
Absent,0	3357	42	3399	0.01236
Present,1	51	445	496	0.10282
Total	3408	487	3895	

PCC = 97.61 %  
 Specificity = 98.76 %  
 Sensitivity = 89.72 %  
 k = 0.892  
 AUC = 0.994

**Classification Tree, Data=BCP,**  
resubstitution accuracies

Actual Class	Predicted		Total
	Absent	Present	
Absent,0	3353	46	3399
Present,1	60	436	496
Total	3413	482	3895

PCC = 97.28 %  
 Specificity = 98.65 %  
 Sensitivity = 87.9 %  
 k = 0.876  
 AUC = 0.967

PCC denotes the percentage correctly classified, sensitivity is the percentage of presences correctly classified, specificity is the percentage of absences correctly classified, k – kappa a measure of agreement between predicted presences and absences with actual presences and absences corrected for agreement that might be due to chance alone, and AUC is the area under the receiver operating characteristic curve. Resubstitution is based on the training set as an estimate of generalization error. Out-of-bag accuracies are based on 10-fold cross-validation in which a random sample is chosen with replacement from the data. Some observations end up in the sample more than once, while others are not included (“out of bag”).

The RF was then used to select important variables to predict the presence of GBBP using the GIS dataset. However, cross-validated percent correct and sensitivities for the five methods are all relatively high and similar. Since the RF percent classified



correct (PCC) was 98.2%, if a different model did not achieve at least this with resubstitution accuracies, it was not used to calculate the cross-validated accuracies. To invoke parsimony, which is to select the model with the fewest assumptions, RF was used to examine the most important variables. Elevation is the most important variable for classification (Table 3), which is expected, as GBBP is only found at high elevations above 2500 meters.

**Table 3**

Variable importance metric for predictor variables from random forests (RF) classifications used for predicting presence of GBBP.

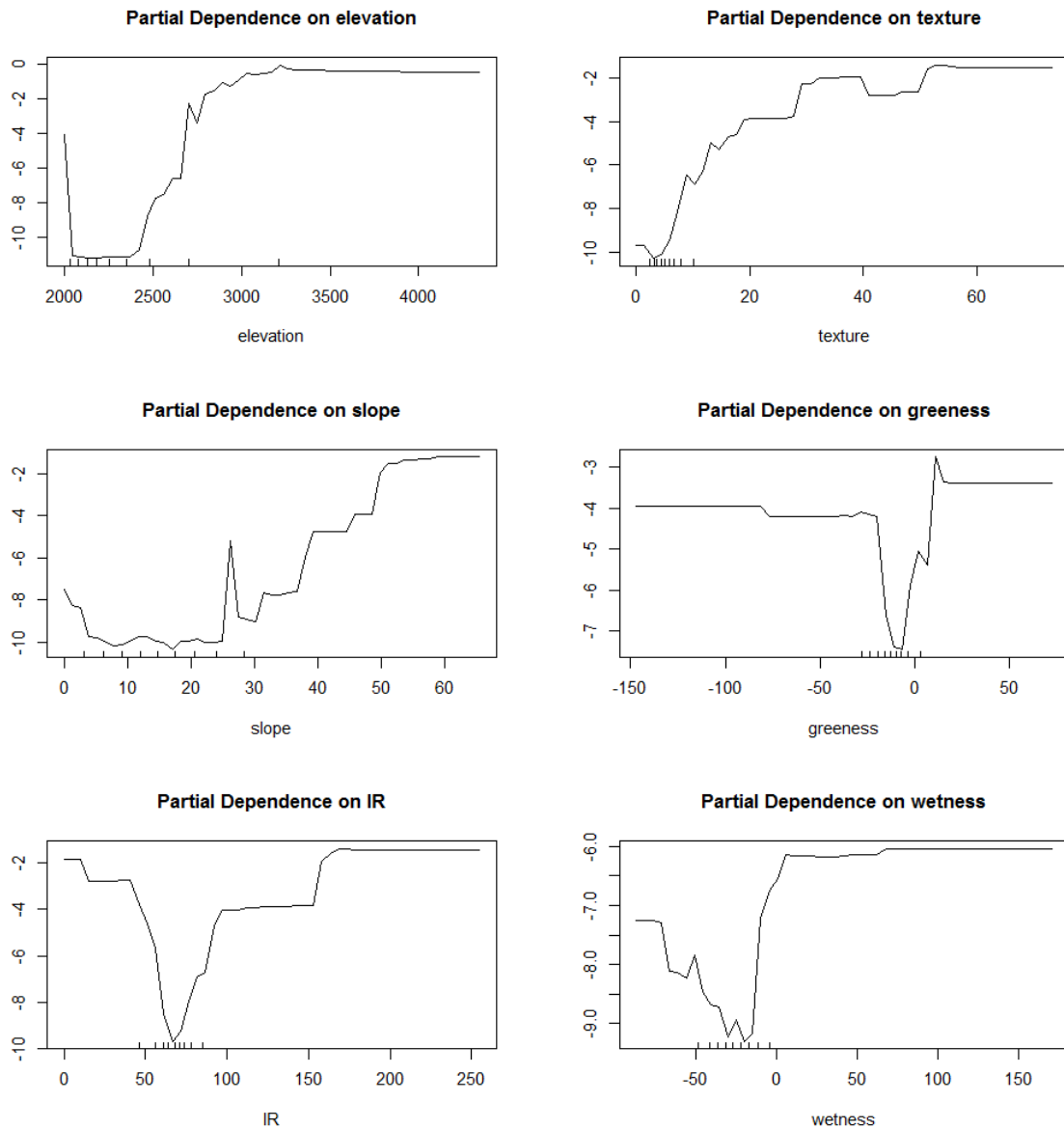
	Mean Decrease Accuracy	Mean Decrease Gini
elevation	69.3	362.5
texture	28.4	45.7
slope	27.2	32.1
NDVI	25.8	38.9
greenness	25.1	24.4
wetness	25.0	35.0
IR	24.6	46.1
aspect	22.8	23.5
brightness	21.6	28.6
blue	19.5	24.5
red	19.0	18.1
green	16.9	17.8

Note: Mean decrease in accuracy (MDA) is the normalized difference of the classification accuracy for the out-of-bag data when the data for that variable is included as observed. Higher values of mean decrease in accuracy indicate variables that are more important to the classification.

In addition to examining the most important predictor variables, partial dependence plots (Fig. 2) characterize relationships between individual predictor variables and predicted probabilities of GBBP presence using RF. These plots visualize the relationship of small numbers of variables in statistical classifiers, such as

classification and regression tools, including RF, GBM, and SVM (Hastie et al., 2002).

Similar variables were used for RF as for the classification tree, which lends credibility to the results. Also, higher texture values are associated with GBBP stands (Fig. 2).



**Fig. 2.** Partial dependence plots for selected predictor variables (6 most important from MDA) for random forest (RF) predictions of the presences of GBBP. Partial dependence is the dependence of the probability of presence on one predictor variable.

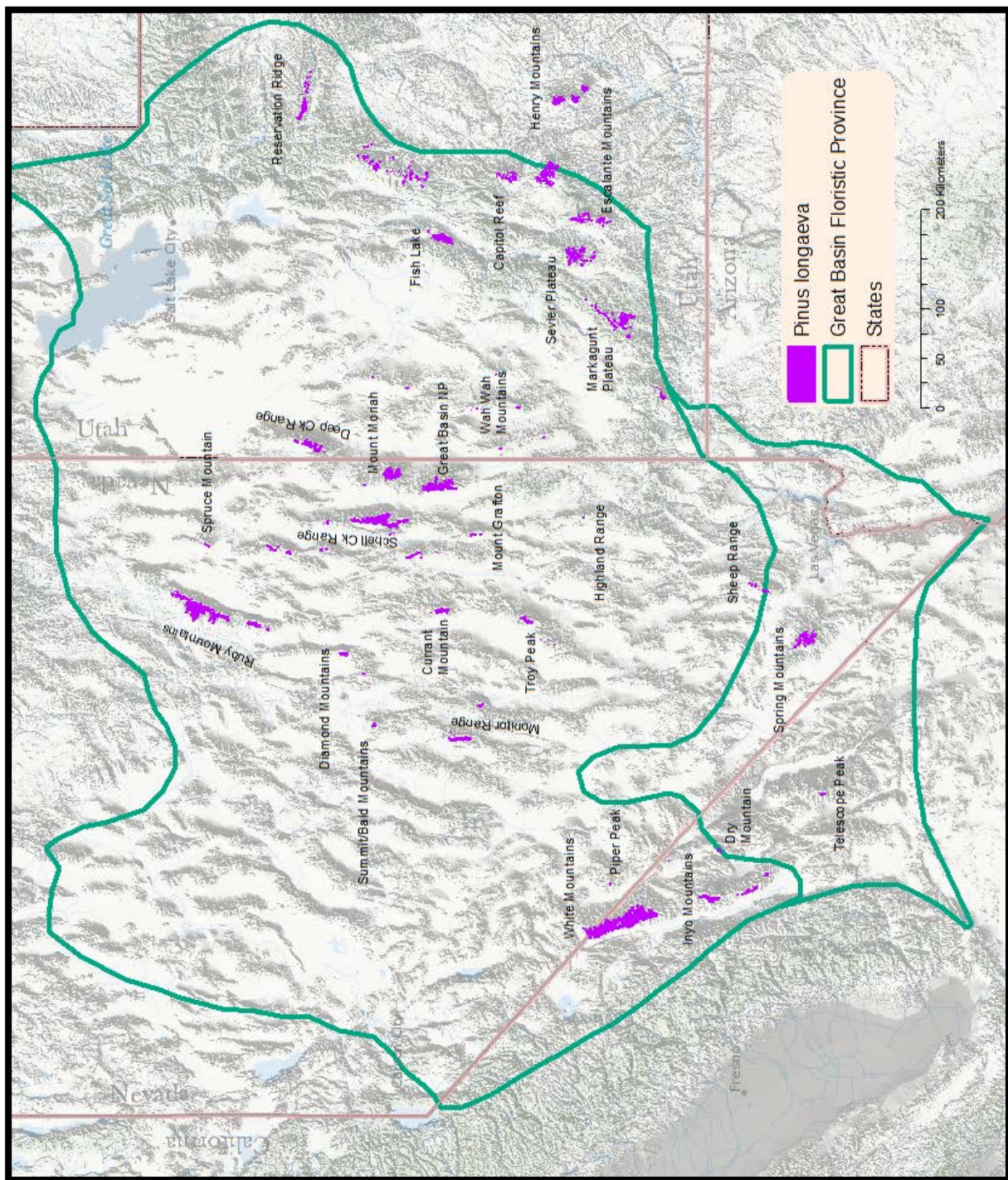
We expect this since GBBP occurs in very sparse stands, often with high contrast to the underlying soil. Higher values of NDVI and IR also are associated with presence of GBBP (Fig. 2). Again, this is logical as it separates forested stands from barren rock.

The data was refit with RF using only the 9 most important independent variables. This changed the overall accuracy of the model from 98.2% correct to 97.6% classified correct (Table 2). This is still exceptionally high accuracy. The final model predicted the presence of GBBP across the entire Great Basin (Fig. 3) using the previously collected GIS terrain and spectral variables. The resulting layer has a much higher spatial resolution (10 meters) than previous mapping projects. Areas on the map represent potential occurrences of GBBP and not actual mapped locations, however this is the best representation of GBBP to date.

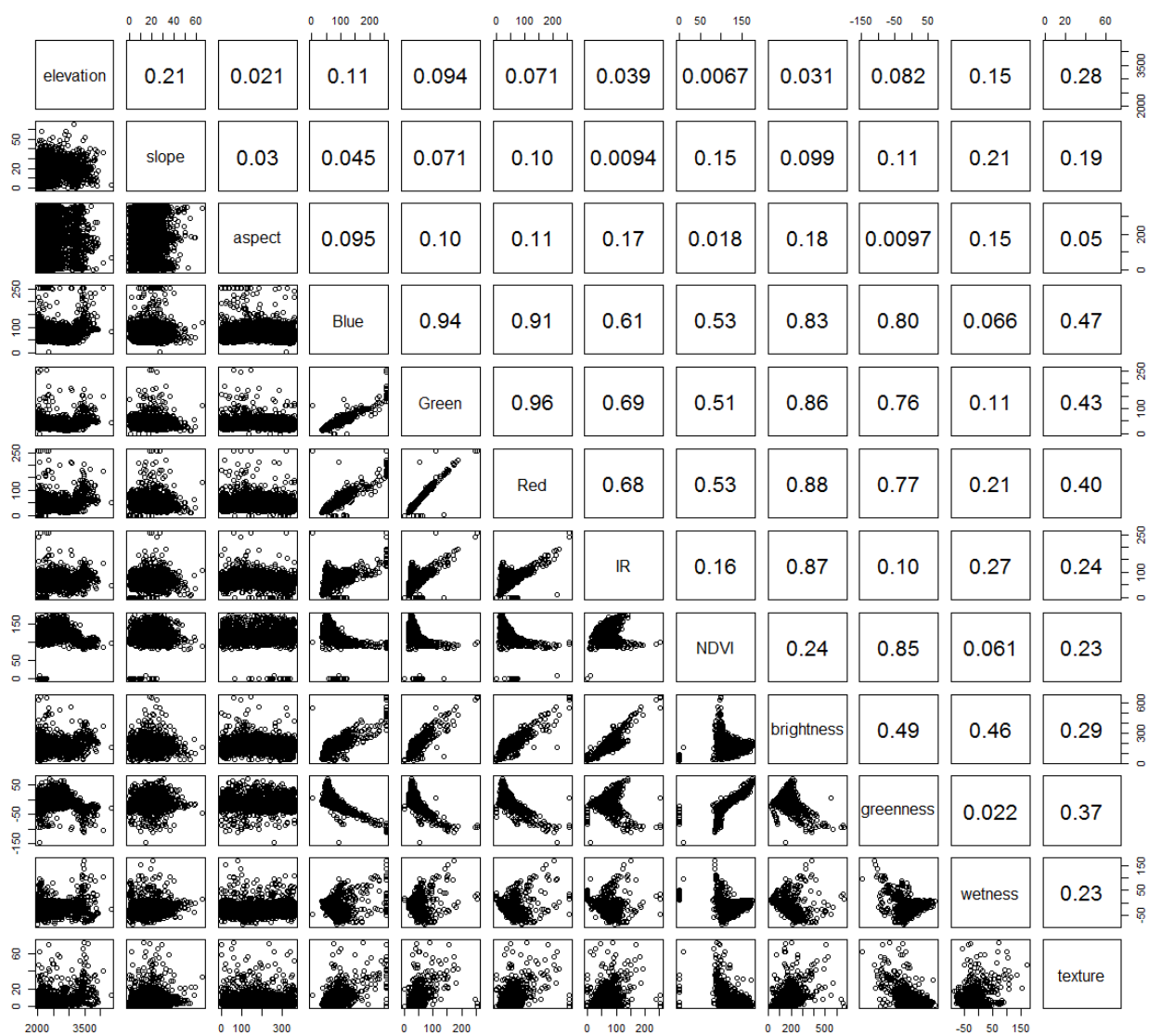
The correlation matrix (Fig. 4) shows that several of the variables, especially the spectral variables, have a high correlation coefficient (over 0.9). However, we should expect some of the variables to be redundant.

#### *4.1. Species Fragmentation*

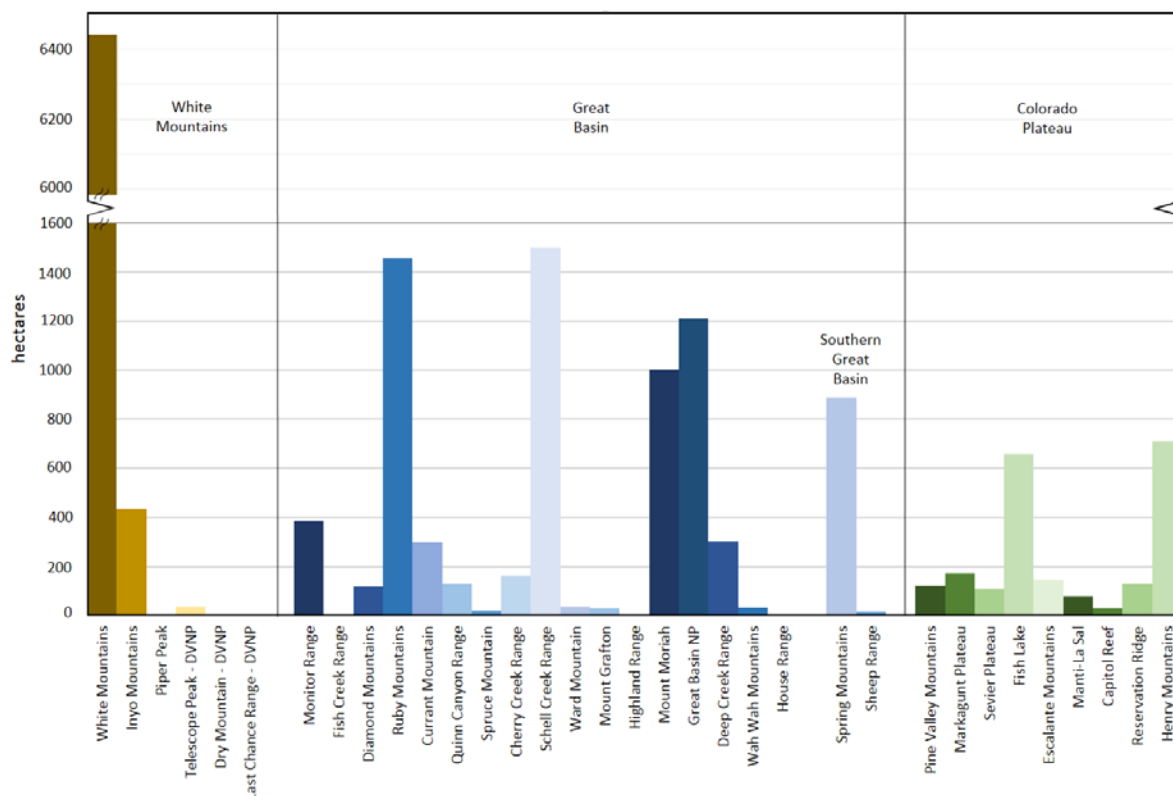
As mentioned earlier, fragmentation can lead to species extinction and fragmentation metrics are useful in assessing risk to the population. From the predicted GBBP GIS map, I have grouped GBBP in 34 groves across three geographic regions, which I have termed the White Mountains, the Great Basin, and the Colorado Plateau stands. The resulting GIS layer contained many isolated pixels that predicted the presence of GBBP. Cells from the GIS were converted to polygons and any occurrence that was not at least three adjacent pixels (300 m<sup>2</sup>) was removed from the layer. While there is the



**Fig. 3.** Species distribution map of Great Basin bristlecone pine (*Pinus longaeva*) from Random Forest model (RF) predicted into the Great Basin Floristic Province, part of the Mojave Desert (south) and in the Henry Mountains (east) where a known stand occurs. Purple polygon line weights were increased for visualization, resulting in areas on map appearing greater than on the ground



**Fig. 4.** Correlation matrix of topographic and spectral variables from Great Basin bristlecone present plots. Correlation coefficient (r-value) in upper right quadrants.



**Fig. 5.** Stand area of Great Basin bristlecone pine. Forest stands have been aggregated using GIS for 34 different ranges across the geographic regions (*White Mountains* = yellows, *Great Basin* = blues, *Colorado Plateau* = greens). Area represents potential occurrences of GBBP and not actual mapped locations.

potential for GBBP to occur at these sites, management plans lend themselves to larger areas. All polygons on each mountain range were tallied together to calculate the distance to the next nearest GBBP stand. The minimum distance between populations ranged from 17.8 km to 72.6 km (mean = 35.0 km, SD = 14.6 km). While there are several well known stands of GBBP that extend over more than 1000 hectares including the Ruby Mountains, Schell Creek Range, Great Basin National Park, and the famous groves in the White Mountains of California (Fig. 5), most stands are less than 400 hectares and occur on isolated peaks and pockets of the Colorado Plateau.

## 5. Conclusions

Although all models tested here classified GBBP with high accuracy as measured by cross-validation and RF out of bag accuracies, RF predicted the occurrence of GBBP with the highest accuracy. Upon visual inspection of the GBBP distribution map, the GBBP model better predicts known locations of GBBP groves than previous distribution maps; however, it is necessary to further validate the accuracy of the model in the field before it is used for critical management decisions. Future work includes examining whether increased spatial resolution of the data used in the model gives a more accurate identification of GBBP stands, or if it is merely a more visually pleasing depiction. To make informed management decisions on a fragmented species such as GBBP, accurate mapping and inventories are necessary. Considering the high accuracy of the RF model, I believe this to be the best map of GBBP to date and this species distribution model will be the foundation to examine large scale changes to GBBP in later chapters.

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## CHAPTER 3

CLIMATE WARMING ALTERS FUELS ACROSS ELEVATIONAL GRADIENTS IN  
GREAT BASIN BRISTLECONE PINE-DOMINATED SKY ISLAND FORESTS<sup>1</sup>**Abstract**

Little is known about fuel characteristics and dynamics in GBBP communities, and current monitoring programs inadequately quantify the surface and canopy fuels of this system. Using the Forest Inventory Analysis (FIA) plot variables of tree species, height, diameter at breast height (DBH), canopy base height (CBH), coarse (CWD) and fine (FWD) woody debris counts, and canopy fuels measurements, this paper examines the effects of climate-induced changes to fuel loading, fire hazard and risk on predicted changes in fire behavior and severity. Field transects were installed using FIA protocols along environmental gradients. Plots were located every 22 chains or ~440 meters along random transects on Mt. Washington in the Great Basin National Park (GBNP) and in the nearby Mt. Moriah Wilderness, NV. Additional plots were installed at Notch Peak (UT), Cave Mountain (NV), and Wheeler Peak (GBNP, NV). Linear regression showed that all classes of FWD decreased with elevation, and only 1000-hr fuels remained constant across elevational transects. This, combined with lower CBH and foliar moisture and increasing temperatures due to climate change, increases fire potential at the Great Basin bristlecone pine treeline, threatening the oldest individuals of this iconic species. New information about discontinuous fuels will aid in management of high elevation alpine treeline forests.

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## 1. Introduction

High elevation five needle pines are declining throughout western North America from climate warming, mountain pine beetle (*Dendroctonus ponderosae* Hopkins), white pine blister rust (*Cronartium ribicola*), and the alteration of naturally occurring fire regimes (Gibson et al., 2008). Climate change effects are especially acute in sky islands, the isolated mountains surrounded by valleys of the Great Basin, as warming temperatures alter tree community distribution and contribute to increased surface fuels. Changing air temperature and precipitation may interact with fire regimes to shorten times to ignition and lower temperatures at ignition from lower moisture content (Gill et al., 1978) of lower elevation populations. Great Basin bristlecone pine (*Pinus longaeva* Bailey) is a high elevation, five needle pine, located near treeline and grows in isolated sky islands of California, Nevada, and Utah. Great Basin bristlecone pine (GBBP) are mainly adapted to survive low-severity surface fires (Zavarin and Snajberk, 1973), however fire-scarred GBBP are found at lower elevations with fire tolerant ponderosa pine (Lanner, 1999). Climate induced changes to the fire regime will alter surface and canopy fuel loading, species composition, fire hazard and risk, and fire behavior and severity on GBBP forests (Schoennagel et al., 2004). Additionally, the amount, arrangement, and continuity of GBBP fuels vary with elevation, community species composition, and time. Fuel loadings are strongly influenced by fire history and site characteristics providing a proxy for temperature change; however, these gradients have yet to be quantified.

### 1.1. Climate change and fire in treeline communities

GBBP are among the oldest organisms on earth. Their distribution is limited to the highest elevations (2700-3700m) in mountain ranges of the Great Basin of the western United States. Because populations are isolated, effects of a warming climate are projected to be particularly acute (Bower et al., 2011). Increasing temperatures are expected to result in pine mortality and introduction of invasive weeds and lower elevation conifers, consequently changing surface fuels composition (Flannigan et al., 2000; Gibson et al., 2008). Historically, fire was thought to be infrequent in GBBP communities at high-elevation sites because stands are open and productivity is low. When fires did occur at high elevations, they were usually small, low-severity surface fires (Bailey, 1970; Bradley et al., 1992). Moisture and climate have more influence on treeline stand dynamics than fire (Lanner, 1988, 1985). Early studies suggested that low tree density and sparse litter in the sub-alpine GBBP forests near treeline did not contain enough fuels to carry fire (Bradley et al., 1992; Lamarche, 1967; Lanner, 1988). At lower elevation sites, the role of historical fire regimes in dictating past stand characteristics and current distribution is not fully known, yet it is likely GBBP experienced a variable fire regime across gradients of site productivity and fuels connectivity. Fuels can be sufficient to carry fire in denser, low-elevation sites where GBBP occurs in mixed forests with limber pine (*Pinus flexilis* James) and/or Engelmann spruce (*Picea engelmannii* Parry) (Bidartondo et al., 2001). GBBP have a low resistance to fire, due to thin bark and low branches and retain their 2.5-3.5 cm needles up to 25-30 years (Bailey, 1970), increasing needle accumulation in crowns and on the surface under GBBP when compared to lower elevation pine species (Jenkins, 2011).

Limited studies of the closely related Rocky Mountain (RM) bristlecone pine (*Pinus aristata* Engelm.) and limber pine communities have found fire scars indicative of frequent, low intensity surface fires in sites that border grassy openings (Coop and Schoettle, 2011). Stand-replacing fire could be the primary disturbance regime for RM bristlecone pine, with a fire return interval of approximately 300 years (Baker, 1992). Evidence suggests that fire severity for RM bristlecone forest types varied through time and space (mixed-severity fire regime) as climate changed at centennial to millennial time scales (Coop and Schoettle, 2011). Additionally, frequent fire presumably played an important role in restricting RM bristlecone pine at lower elevations in pre-settlement times (Coop and Schoettle, 2011). Physical limitations on forest structure, such as age and density, affect the accumulation of surface fuels and crown fuels. For example, a study from boreal forests in Finland indicates a site's disturbance history is the determining factor for fuels quantity and decay class distribution (Aakala, 2010). While Baker (1992) found that stand-replacing fires in RM bristlecone pine initiated regeneration, little is known of post-fire succession in mixed-conifer forests containing GBBP. It is important to understand the fire history of GBBP/limber pine/Engelmann spruce and other montane forests in the Great Basin to develop appropriate adaptation strategies for managing these systems with a warming climate.

Most wildfire and fuel models were designed for vegetation types that burn frequently, are characterized by continuous surface fuels, or are of interest to fire management (Rothermel 1972). Thus, discontinuous fuel associated with GBBP are not represented by traditional fuel models, and might be more similar to heterogeneous systems like piñon juniper woodlands. Extensive characterization of piñon juniper

woodlands have examined the sparse surface fuels and discontinuous tree canopies that curtail fire spread under low wind conditions (Floyd et al., 2008; Huffman et al., 2009; Miller et al., 2000; Romme et al., 2009). One physics-based fire model was applied to piñon juniper woodlands and results suggest sparse fuels in heterogeneous forests propagate fire because dead needles on the ground provide surface fuels, and allow increased winds through the canopy and sub-canopy (Linn et al., 2013). A fire behavior study in arid vegetation communities in Australia developed models to predict the sustainability of fire spread, fire type (surface or crown), rate of spread and flame height in a discontinuous fuel type (Cruz et al., 2013). They found that sustainability of fire spread was a function of litter fuel moisture with wind speed having a secondary but still significant effect. The continuity of fine fuels was also significant. Initiation of crown fire was primarily determined by wind speed. Cruz et al. (2013) presented the need to find threshold conditions for sustained fire propagation based on wind speed and fine fuel moisture content. While fire spread models could be helpful for assessing fuel changes in GBBP, validation data are unavailable, and therefore are outside the scope of this study.

### *1.2. Environmental gradients*

Environmental gradients relate factors such as elevation, temperature, water availability, light, and soil nutrients, or their closely correlated surrogates. Forest composition usually changes along environmental gradients in predictable ways (Peet, 2000). For example, elevation is often a surrogate variable which approximates changes in temperature and moisture (Peet, 2000). At lower elevations, moisture and temperature may allow for a forest to reach full crown closure, although a mid-elevation site might

not reach full crown closure. At upper alpine treeline (the edge of the habitat at which trees are capable of growing, found at high elevations and in frigid environments) tree density and decomposition is typically limited by a short growing season. However, high severity disturbances are rare (low frequency and high intensity) allowing for large tree size diversity (Miller, 1997).

### *1.3. Fuels composition across environmental gradients*

Understanding how fuels structure and composition varies across environmental gradients in Great Basin sky islands is necessary to predict how fire frequency and intensity may change at high elevations with a warming climate. Studies that have modeled severity and length of forest fire season employing general circulation models (GCMs) have estimated that seasonal severity ratings may increase by 10–50% over most of North America, (Flannigan et al., 2000) suggesting that fire is an predominate agent of change and has the potential to overshadow direct effects of climate change on species distribution and migration. Our best tool at estimating the potential fire intensity of vegetation communities, or the amount of energy released during a fire, is fine and coarse woody debris surveys (Brown, 1974), yet fuels vary greatly depending on topography, meteorological influences, fuel type and characteristics of previous disturbance. Warming temperatures, lower humidity, and lower fuel moisture increase the potential for high severity fires (Abatzoglou and Williams, 2016; Littell et al., 2016). Research is needed that will help managers plan for transitions to new conditions and habitats, manage migrations along expected climatic gradients, prepare for higher-elevation insect and

disease outbreaks, and anticipate forest mortality events and altered fire regimes (Millar et al., 2007).

The objective of this paper is to quantify spatially discontinuous fuel structure across changing environmental gradients in GBBP stands. This information is useful to understand how climate change affects the fire regime and GBBP health and abundance. We assume that elevation is a surrogate for warming air temperatures. To understand how global climate change will alter wildland fuels, we quantified differences in GBBP fuels and how fuels differ across elevation gradients. We compare the relationship between forest structure and environmental gradients to predict changes in surface and canopy fuels of GBBP communities with increasing temperatures. A comprehensive stand assessment and fuel survey of this iconic species provides a foundation upon which management decisions and dialogue can be based. Consequently, this research is valuable for forest and fire planning and management, as well as prioritization and design of restoration efforts and climate change adaptation strategies.

## **2. Study Site**

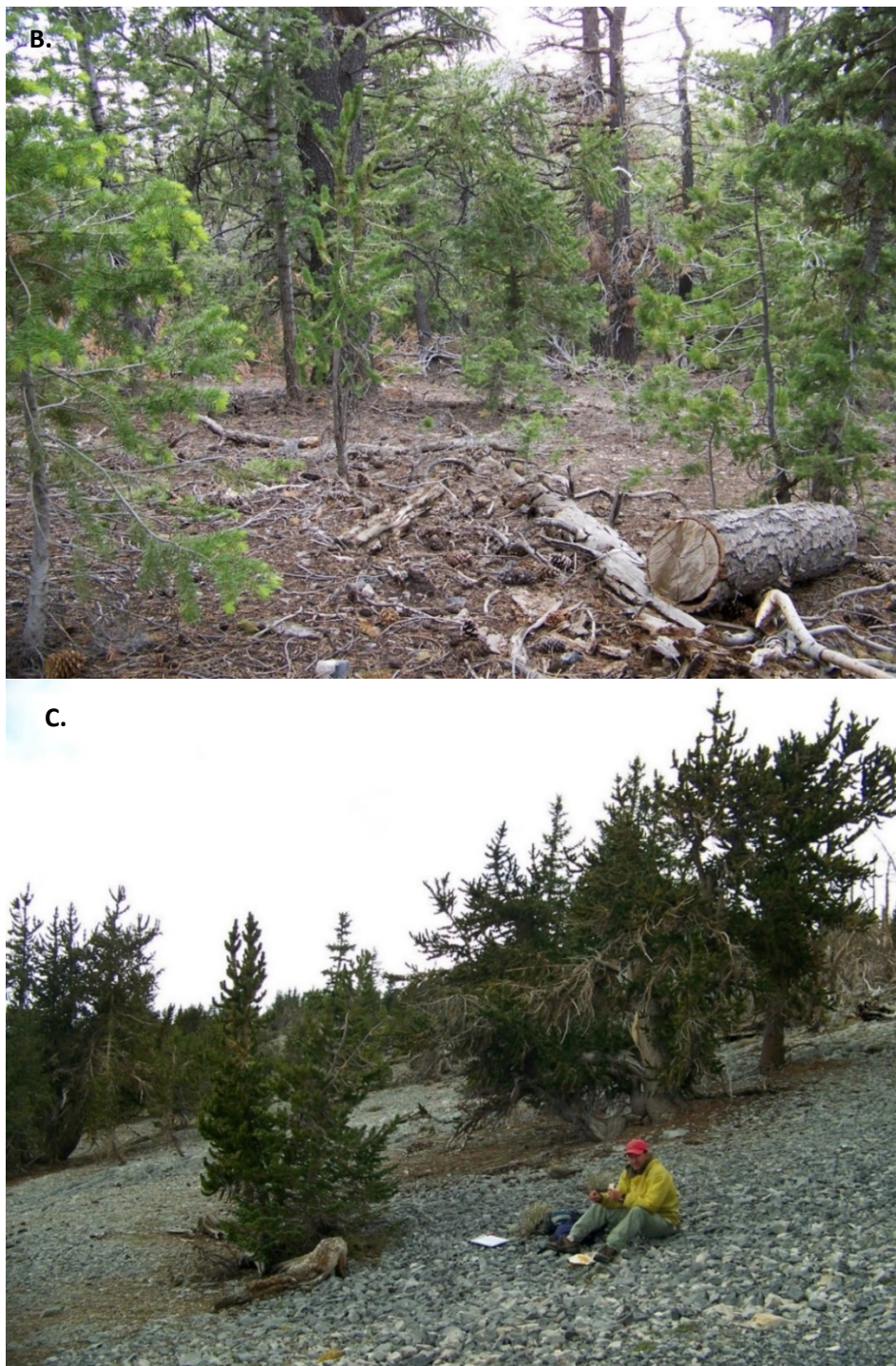
The geographic extent of sampling was limited to sky islands of the Great Basin of Nevada and western Utah. Sample sites were at Washington Peak, NV (38.90°, -114.31°, 3,475 m), Wheeler Peak, NV (39.00°, -114.30°, 3,415 m), and Mt. Moriah, NV (39.29°, -114.20°, 3,300 m). Additional individual plots were installed at Cave Mountain, NV (39.16°, -114.61°, 3,230 m), and Notch Peak, UT (39.14°, -113.40°, 2,800 m).

The Great Basin of California, Nevada, and Utah has an arid climate characterized by Basin and Range topography, with numerous high mountain peaks and low



intermountain valleys. Upper treeline is approximately 3,300 m and lower treeline is approximately 2,100 m (Thompson and Mead, 1982). Mountains and basins create steep environmental gradients, which greatly influence the composition and structure of vegetative communities (Peet, 2000). As mentioned, fires are infrequent in high elevation GBBP forest due to sparse fuels (Fig. 1C). Yet this is not the case at the lower treeline of GBBP communities, which are dominated with shrubs and other forest species (Fig. 1A). The mid elevation sites are typical of mixed conifer forests (Fig. 1B).





**Fig. 6.** Fuels complexes of Great Basin bristlecone pine (GBBP) communities. (A) Lower elevation communities have higher fuels from denser vegetation and shrubs. (B) Mid elevations have less fuels than lower elevations, but retain considerable 1 hr, 10 hr, and 100 hr fuels. (C) Fuels in the highest elevations near treeline are mostly litter and duff under individual trees.

### 3. Methods

#### 3.1. FIA data and sampling

Data were from a combination of US Forest Service Forest Inventory and Analysis (FIA) program plots (O'Connell et al., 2015) that contained GBBP and additional study sites that were selected using expertise from local US Forest Service and National Park Service personnel. The FIA variables collected at each plot were tree species, height, diameter at breast height (DBH), canopy base height (CBH - the vertical distance between the surface fuels and live canopy fuels), seedling and sampling counts, and surface fuels measurements. All 34 plots that contained GBBP in the FIA database from Utah, Nevada and California were examined for forest structure and fuel loading. The FIA program uses a three phase sampling design covering all public and private land in the United States (Bechtold and Patterson, 2005; O'Connell et al., 2015). In Phase 1, remote sensing and aerial photography classify forested lands, percent tree cover, and forest use (Bechtold and Patterson, 2005). In Phase 2, four 7.3 m fixed-radius subplots spaced 36.6 m apart in a triangular arrangement with subplot 1 in the center and subplots 2, 3, and 4 at azimuths of 0°, 120°, and 240°, respectively, from the center of subplot 1 measure tree species, size, and density (O'Connell et al., 2015) (Fig. S1-B). Phase 3 measurements assess forest health such as tree crown, soil, and lichen conditions, and down woody material (Bechtold and Patterson, 2005; Woodall and Monleon, 2010) and are not yet available in much of the U.S.

Due to the relatively small sample of FIA plots with GBBP, 76 additional plots using FIA Phase 2 protocols were installed along elevational gradients to assess changes

in GBBP communities (total plots = 110). From the lowest elevation plot (2827 m), subsequent plot centers were located by pacing approximately 400 m up the fall line (mean distance between plots from GIS = 418 m) (Fig. S1-A). If no trees were located at the 400 m interval, this point was skipped. Survey plots are fixed radius plots, which were modified FIA plots (Fig. S1-B), each containing four subplots. Every plot contained at least one GBBP. Other species included limber pine (*Pinus flexilis* James), Engelmann spruce (*Picea engelmannii* Parry), subalpine fir (*Abies lasiocarpa* var. *latifolia* Nutt.), Douglas-fir (*Pseudotsuga menziesii* var. *glauca* Beissn), aspen (*Populus tremuloides* Michx.), piñon pine (*Pinus edulis* Engelm.), juniper (*Juniperus spp.*), mountain mahogany (*Cercocarpus ledifolius*), and ponderosa pine (*Pinus ponderosa* Douglas).

### 3.2. Fuels sampling

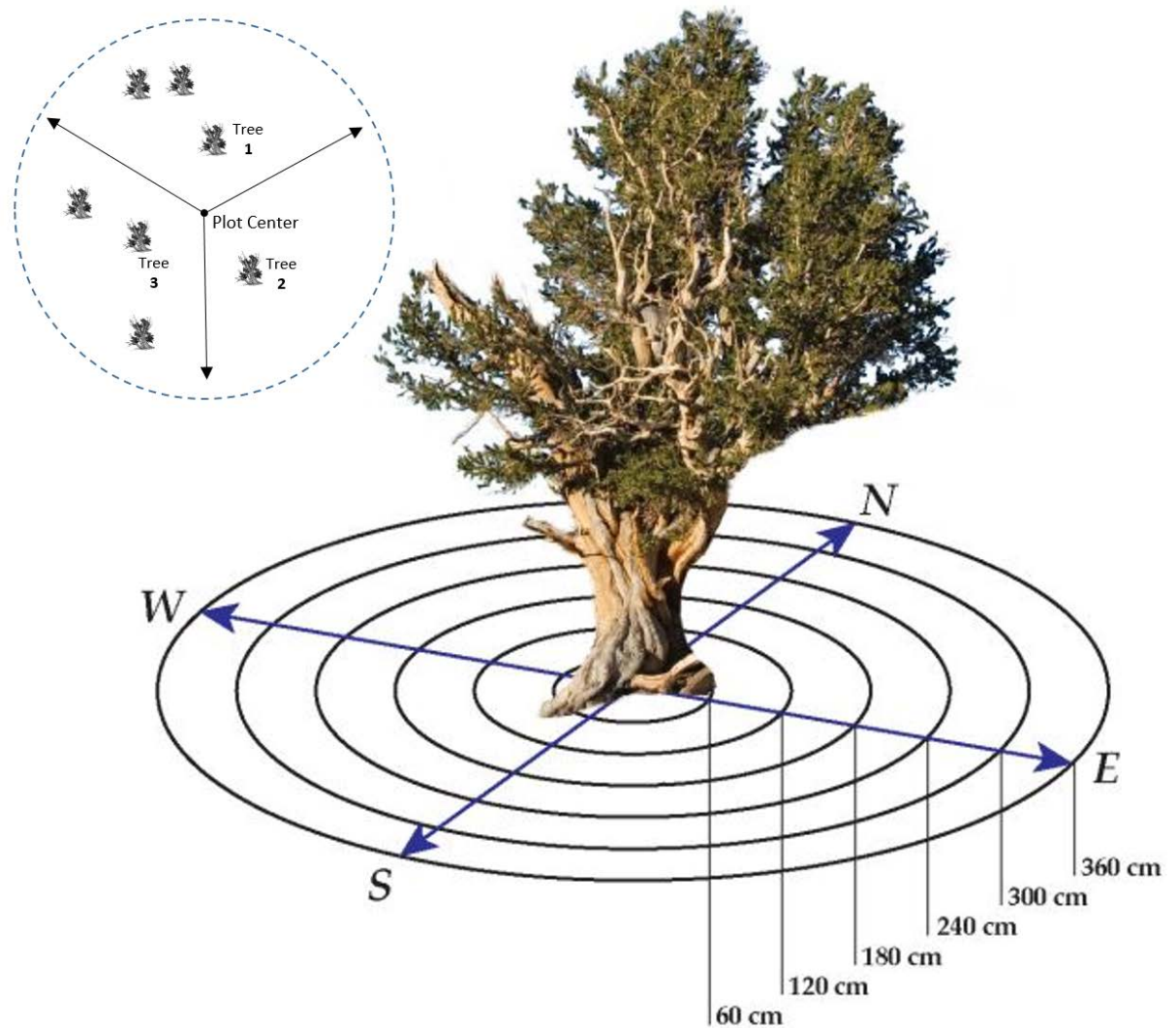
We quantified fuel loading, specifically the dry weight biomass of fuel per unit area ( $\text{kg m}^{-2}$ ) of four major surface fuel components: litter (freshly fallen non-woody material which includes leaves and cones), duff (partially decomposed biomass whose origins cannot be determined), fine woody debris (FWD; 0–7.6 cm diameter), and coarse woody debris (CWD, >7.6 cm diameter; commonly referred to as logs or 1000 hr fuels). Estimates of CWD biomass are often used for large-scale fire/fuel and carbon monitoring efforts while FWD comprise a substantial portion of fuel loadings and, to a large extent, determine fire behavior (Burgan and Rothermel, 1984). Down woody material, which includes twigs, branches, stems, and tree boles in and above the litter was sampled using Brown's method (also called the line-intersect or planar-intersect method) (Brown, 1974). This transect method is used by FIA and Fire Ecology Assessment Tool/Fire Effects

Monitoring and Inventory System (FEAT/FIREMON) Integrated (FFI) programs (Lutes et al., 2009). Brown's method samples both fine and coarse woody material by diameter class by counting and measuring the size of all woody pieces in the vertical sampling plane. The diameter classes correspond to the 1 hr (<1cm), 10 hr (1-2.5cm), 100 hr (2.5-7.6cm), and 1,000 hr (>7.6cm) fuel classes used in the U.S. National Fire Danger Rating System (Deeming et al., 1977). Tallies of 1 hr and 10 hr fuels were made along the distal 1.83 m of the fuels transects, while 100 hr fuels were made on 3.05 m, and 1000 hr fuels were made on 7.32 m of the transect (Fig. S1-B). Volume and weight were calculated by applying estimates of the woody material's specific gravity following Brown (1982).

### *3.3. Tree-specific fuels sampling*

Jenkins (2011) described the distribution of surface and aerial fuels under whitebark pine (*Pinus albicaulis* Engelm.) forests which are similar to other alpine treeline five-needle pine forests. He defined the pine fuel zone as fuels lying within the drip line of a tree and the non-pine fuel matrix as the area between adjacent trees. While installing our fuels transects, we observed similar surface and aerial fuel distribution in GBBP communities as whitebark pine forests (Fig. 6C). In high elevation GBBP stands, we expect that fuel influence on surface fire behavior will be a function of the distribution of pine fuel zone and non-pine fuel matrix. The pine fuel zone will increase as stand density increases and may compose 100% of the surface fuel matrix in very dense stands (Jenkins, 2011). To quantify this unique patchy and discontinuous fuel complex, we measured litter and duff of 105 trees in the four cardinal directions (NSEW) under three trees from each plot (Fig. 7). From the plot center, the closest GBBP tree

within the northern third ( $300^{\circ}$ - $60^{\circ}$ ), south-eastern third ( $60^{\circ}$ - $180^{\circ}$ ), and south-western third ( $180^{\circ}$ - $300^{\circ}$ ) were selected for sampling. In each cardinal direction away from the sample tree bole, litter and duff depth were measured at 60 cm intervals until the end of the tree crown (Fig. 7).



**Fig. 7.** Sample design for individual tree surface fuels. From the plot center (inset) the closest GBBP tree within the northern third ( $300^{\circ}$ - $60^{\circ}$ ), south-eastern third ( $60^{\circ}$ - $180^{\circ}$ ), and south-western third ( $180^{\circ}$ - $300^{\circ}$ ) of plots were selected for sampling. Litter and duff were measured in the four cardinal directions (NSEW) under the three trees. In each cardinal transect, litter and duff depth were measured at 60cm intervals until the end of the tree crown was reached.

### *3.4. Fuels cover and continuity*

While there is a large shrub component (up to 90%) in some of the lowest elevation occurrences of GBBP (Fig. 1A), shrubs are rare or absent in most stands. Fuels cover is important because it describes the size of the fuel gaps that limit fire propagation. We assessed the relative amounts of fuels continuity by utilizing Landsat satellite images. The August 24, 2012 Landsat-7 Enhanced Thematic Mapper (ETM+) image was chosen because it was the cloud-free image closest to the dates of field sampling. The spectral indices Normalized Difference Vegetation Index (NDVI), Brightness, and Greenness estimate fuels cover and continuity. NDVI is a graphical indicator to assess whether a pixel contains live green vegetation. It is based on the ratio of the red and near-infrared (NIR) spectral values, computed as  $NDVI = (NIR-RED) / (NIR+RED)$  (Xavier and Vettorazzi, 2004). Brightness is an estimate of bright or dark soil values, and greenness is a measure of green vegetation. Brightness and Greenness are the first two components of a tasseled cap transformation (Huang et al., 2002), a method for enhancing spectral information content of Landsat TM data. Landsat pixels represent a uniform 30 by 30 m spectral sample, a larger spatial extent than our field plots, which gives a broader overview of fuels continuity. We intersected field plots with the calculated indices of the Landsat pixel. When our field plots were at the margins of pixels, we averaged the values for all pixels that intersected a plot.

### *3.5. Canopy fuels*

Canopy fuels are the main fuel layer supporting crown fire spread (Cruz et al., 2003). Canopy base height (CBH) is the vertical distance between the surface fuels and

live canopy fuels. CBH influences the likelihood of crown fire initiation and the interaction between combustion of surface and canopy fuels (Cruz et al., 2003). We collected CBH at 16 plots and used this and other forest structure variables such as DBH and tree height, to calculate several variables to assess the canopy structure and crown fire potential of GBBP stands. Canopy length (CL), the average length of the canopy fuel stratum, was calculated by subtracting CBH from tree height and averaging it over the stand. Canopy fuel load (CFL) in  $\text{kg m}^{-2}$  was estimated using the allometric foliage weight equations from Brown (1978). As mentioned in Cruz et al. (2003), no published allometric equations were found for GBBP, so whitebark pine was used as a surrogate based on similarity in the tree crown structure. Canopy bulk density (CBD) in  $\text{kg m}^{-3}$ , is a measure of how closely canopy fuels are packed. It reflects the likelihood that fire can move through the forest and was calculated as  $\text{CFL/CL}$  (Cruz et al., 2003). Stand density ( $\text{trees ha}^{-1}$ ) was calculated using FIA tree expansion factors (US Department of Agriculture, 2007) as an estimate for inter-crown distance (ICD). ICD is the distance from one tree crown to an adjacent tree crown and is less in high-elevation five-needle pine stands compared to stands of other pine species with similar basal areas (Jenkins, 2011).

### *3.6. Foliar moisture content*

To assess live foliar moisture content (FMC), needles were randomly selected from four GBBP trees at three different elevations (low = 2640 m, mid = 2910 m, high = 3160 m) during the first week of July, August, and September, 2012 ( $n = 36$ ).

Approximately 20 g of live needles from each sample were weighed to the nearest 0.01 g



and then oven-dried at 105° C for 48 hours and reweighed to obtain a dry weight (Matthews, 2010). Samples were kept frozen until processed. FMC was computed as the percentage of the oven-dry weight to dry foliage weight.

### 3.7. Data analysis and statistics

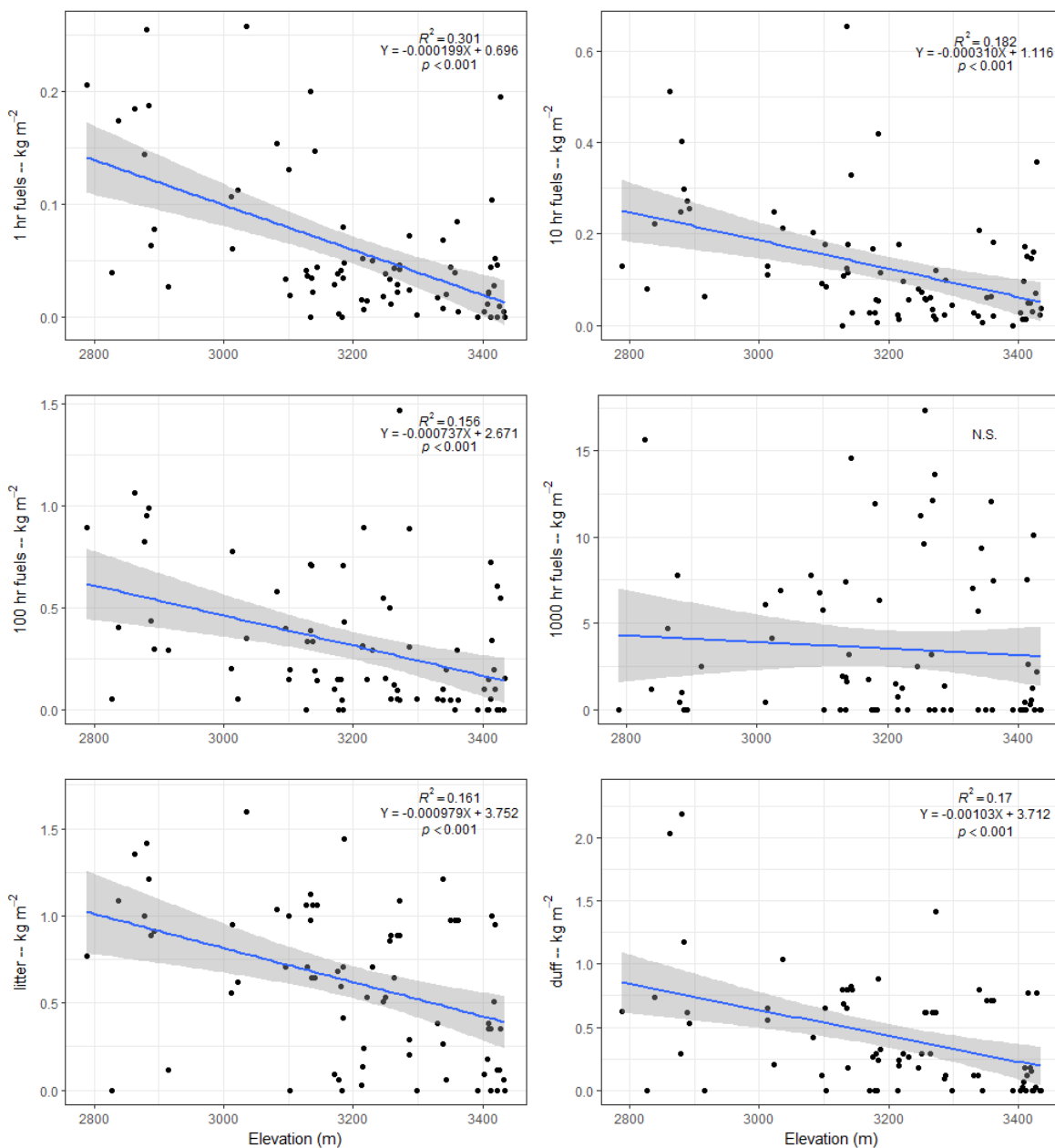
Forest floor CWD and FWD transect counts were converted to weight of fuel per unit area ( $\text{kg m}^{-2}$ ) following Brown (1982). Litter and duff weight per unit area ( $\text{kg m}^{-2}$ ) was estimated from depth measurements by using the equation developed for foxtail pine (*Pinus balfouriana* Grev. et Balf.), a close relative to GBBP (van Wagtenonk et al., 1998). Regression coefficients via generalized linear models (GLM) were developed relating forest floor mass to elevation. To characterize surface fuels dissimilarity along environmental gradients, a non-metric multi-dimensional scaling (NMDS) ordination based on a matrix of Euclidean dissimilarities was calculated on FWD, CWD, litter and duff amounts. NMDS collapses information from multiple dimensions to fewer dimensions, so that data can be visualized and interpreted (McCune et al., 2002) (Fig. S3 in supplemental information). Stand densities in trees  $\text{hectare}^{-1}$  were calculated for each plot using the tree expansion factors (coefficient used to scale each tree on a plot to a per-area basis) in the FIA user manual (O'Connell et al., 2015). Regression coefficients were also calculated for stand density ( $\text{trees ha}^{-1}$ ) and height to live crown for the same elevational gradients. Post hoc mean comparisons using Tukey-Kramer tests were used when a significant difference among elevation class was identified in canopy fuels. A generalized linear model (GLM) with a negative binomial link was fit to the litter and duff measurements made in the four cardinal directions under the sampled individual

trees. We used the negative binomial distribution because data with many zero values cause over-dispersion, or greater variability than would be expected. GLMs are mathematical extensions of linear models that do not force data into unnatural scales, and thereby allow for non-linearity and non-constant variance structures in the data (Hastie and Tibshirani, 1990). The negative binomial distribution generates realistic heterogeneity representative of spatial clustering of individuals and other small-scale processes (Bolker, 2008). All statistics were completed using R statistical software (R Development Core Team, 2015).

## **4. Results**

### *4.1. FWD and CWD fuels loading*

The mean weight of fuel per unit area ( $\text{kg m}^{-2}$ ) for low (2700–3000m), mid (3000–3300m), and high (3300–3500m) elevation classes are reported in Table 4. As elevation increases, FWD and CWD decrease, limiting the surface fuels available to carry a fire. Separate regression coefficients were calculated for each FWD and CWD class (Table 5, Fig. 8) by elevation. All slope coefficients were significant ( $p < 0.001$ ) except for the largest class (1000-hr fuels). All FWD classes, litter, and duff are highly correlated. CWD is not strongly correlated to any other classes (Fig. S2). NMDS ordination of the fuels classes is characterized by high linear fit ( $R^2 = 0.99$ ) and a low stress value (0.058) indicating a good representation of all variables in two dimensions. The ordination was highly correlated with elevation and slope indicating that all measurements of fuels (except CWD) change with elevation (Fig. S3).



**Fig. 8.** Coarse and fine woody debris (CWD and FWD), litter and duff in kg m<sup>-2</sup> versus elevation in Great Basin bristlecone pine communities. Regression coefficients for all fuel sizes except 1000-hr fuels were significant ( $p < 0.001$ ) including litter and duff. Shaded areas are the 95% confidence intervals.

**Table 4**

Mean weight of fuel per unit area ( $\text{kg m}^{-2}$ ) and standard error ( $\text{kg m}^{-2}$ ) for fine woody debris (FWD), coarse woody debris (CWD), and litter and duff fuel at low, mid and high elevations. (Litter and duff depth measured in mm in parenthesis).

Elevation		1hr	10hr	100hr	1000hr	Litter (mm)	Duff (mm)
Low	Mean					0.85	18.6
		0.12	0.21	0.49	3.94	(9.6)	(7.4)
	SE	0.02	0.03	0.08	0.96	(1.2)	(1.6)
Mid	Mean					0.62	12.0
		0.04	0.10	0.32	3.79	(7.1)	(4.8)
	SE	0.01	0.02	0.06	0.90	(0.7)	(0.7)
High	Mean					0.43	7.2
		0.03	0.08	0.15	2.77	(4.8)	(2.9)
	SE	0.01	0.02	0.04	0.79	(0.9)	(0.7)
All	Mean					0.61	12.1
		0.06	0.12	0.31	3.50	(6.9)	(4.8)
	SE	0.01	0.01	0.04	0.53	(0.6)	(0.6)

**Table 5**

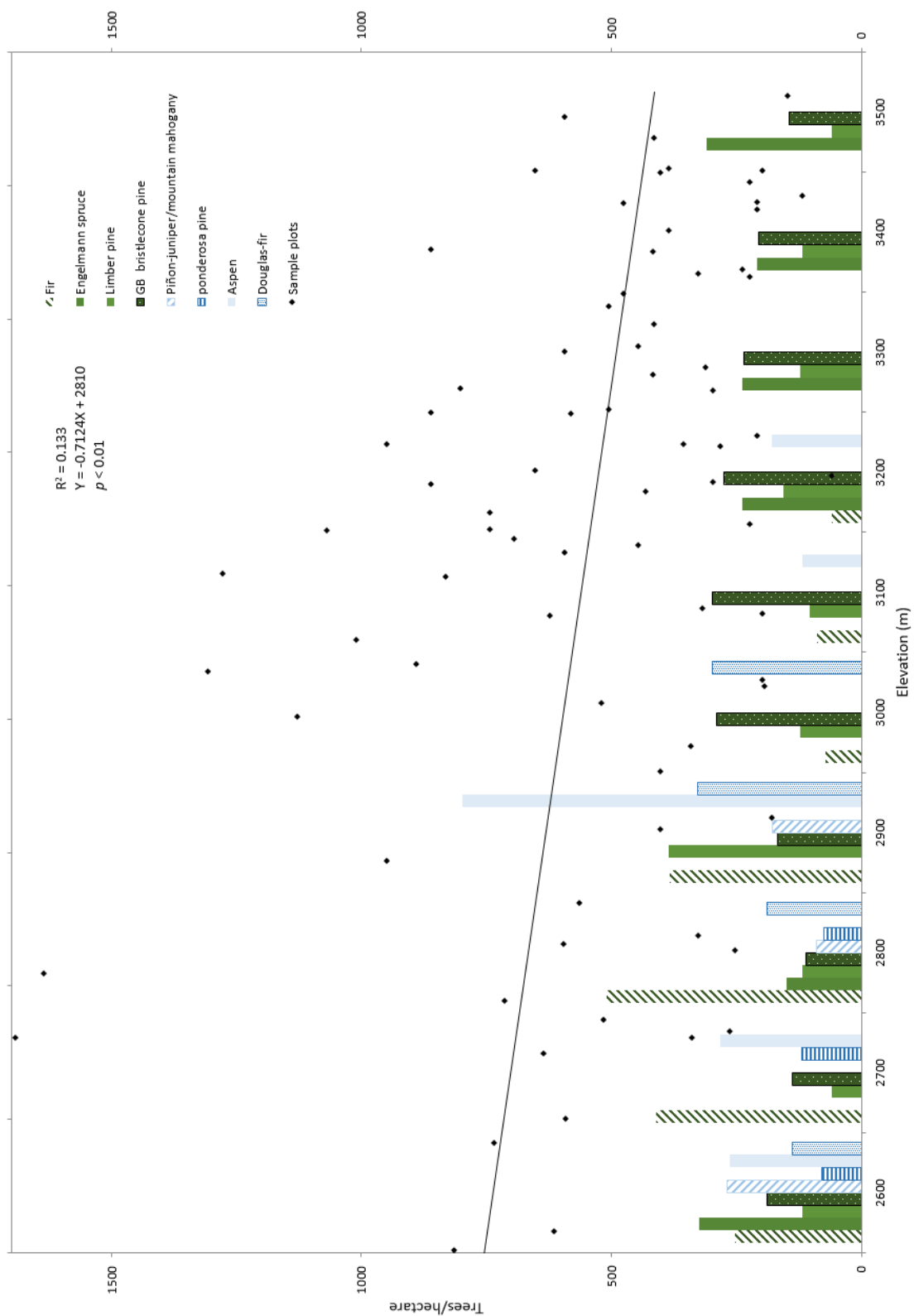
Regression coefficients of fuel per unit area ( $\text{kg m}^{-2}$ ) weight for FWD, CWD, litter and duff fuel classes by elevation. N.S. = not significant.

Fuel class	Slope	Intercept	<i>p</i> -value	Sig-level	R <sup>2</sup>
1-hr	-0.0002	0.696	<0.001	***	0.301
10-hr	-0.0003	1.116	<0.001	***	0.182
100-hr	-0.0007	2.671	<0.001	***	0.016
1000-hr	N.S.	N.S.	N.S.	N.S.	
litter	-0.0010	3.75	<0.001	***	0.161
duff	-0.0010	3.71	<0.001	***	0.170

#### 4.2. Stand density, fuels cover, and community composition

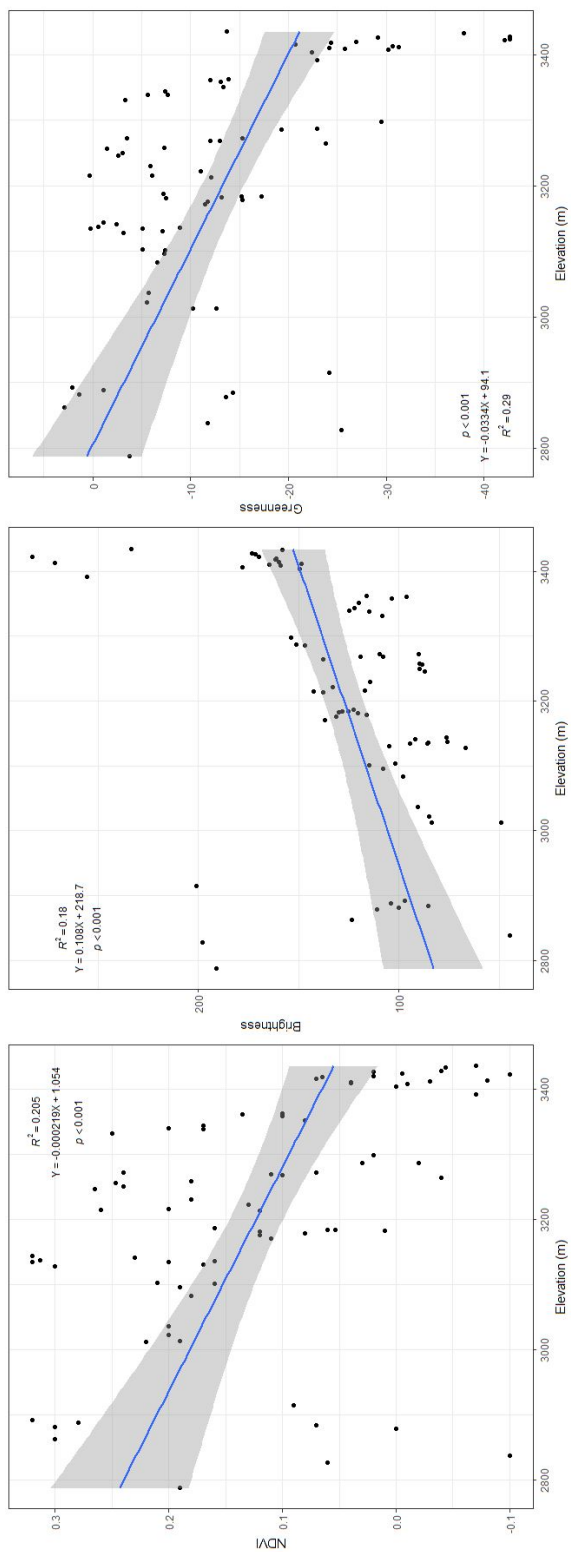
GBBP stand density ( $\text{trees ha}^{-1}$ ) also significantly decreased with elevation (Fig. 9). Stand density is an important contributor to the continuity of surface and aerial fuels (Cruz et al., 2003). Only 13% of variability is explained by the regression model ( $R^2 = 0.133$ ), likely due to only examining stands that have at least one GBBP in the plot. If

nearby forest stands at mid elevations (2600-3000 m) were included, there would likely be higher stand densities. Stand density index (SDI) (Reineke, 1933; Shaw, 2000), describes density that is sensitive to the diameter of the trees, and was calculated for all trees and for each plot. The SDI was not significant, however SDI is best used as a summary variable in even aged stands (Shaw, 2000). SDI is less applicable to long lived GBBP communities, in which very old trees may have a relatively large DBH with little live crown compared to more typical timber producing conifer species. The number of species that make up forest communities also decreases with elevation, from up to 8 dominate tree species at lower elevations to mostly GBBP, limber pine and Engelmann spruce at upper alpine treeline. At lower and mid elevations, GBBP makes up only a minor component (7-14%) of the mixed forest community, growing with other lower elevation tree species including white and subalpine fir, piñon/juniper species, ponderosa pine and Douglas-fir (Fig. 9).



**Fig. 9.** Stand density (trees hectare<sup>-1</sup>) versus elevation. Points are density at each individual plot with actual elevation, bar-graph is species density parsed into 100 m bins.

More important than fuel loads are the size of the fuel gaps that limit fire propagation. The results from the Landsat indices of vegetation cover reiterate the findings from CWD and FWD sampling. As elevation increases, NDVI and Greenness decrease, indicating less vegetation and fuels available to carry a surface fire. Conversely, as elevation increases, Brightness (a measure of exposed soil) also increases, indicating larger gaps between trees, or less continuous fuel cover (Fig. 10).

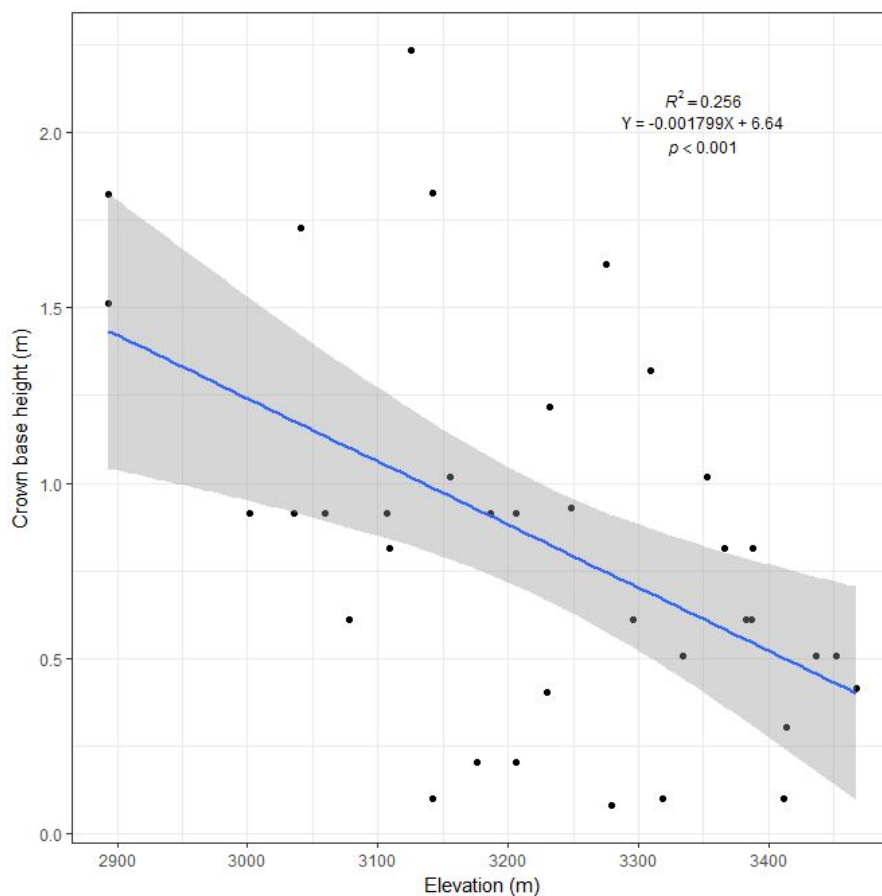


**Fig. 10.** NDVI, Brightness, and Greenness (Landsat-7 ETM+, August 24, 2012) versus elevation in Great Basin bristlecone pine communities. Regression coefficients were all significant ( $p < 0.001$ ). Shaded areas are the 95% confidence intervals.



### 4.3. Canopy fuels

Canopy base height declined significantly with elevation ( $p < 0.001$ ,  $R^2 = 0.74$ ) (Fig. 11). As elevation increased, the branches of GBBP were closer to the ground, which could facilitate fire into the crown of the trees. All crown fuels metrics vary by elevation (Table 6). Tree height, CBH and crown length (CL) decreased with increasing elevation, while available crown fuel load (CFL) and crown bulk density (CBD) increase with elevation (Table 6).



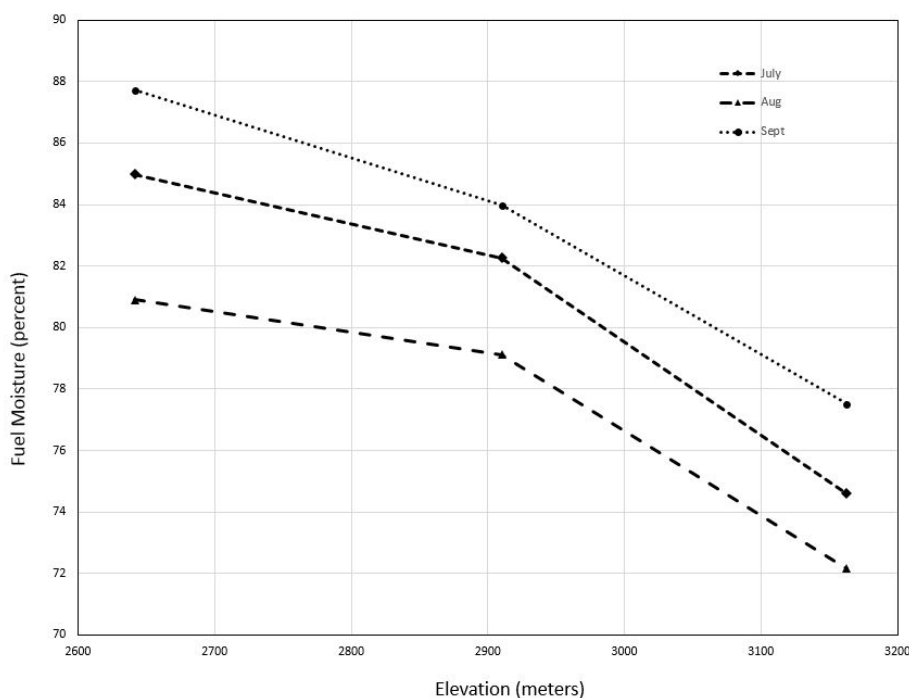
**Fig. 11.** Great Basin bristlecone pine crown base height (CBH) (the distance from the ground to the first tree branches) versus elevation. Shaded areas are the 95% confidence intervals.

**Table 6**

Great Basin bristlecone pine canopy fuels: tree height (Ht), canopy base height (CBH), diameter at breast height (DBH), Canopy length (CL), Canopy fuel load (CFL), and Canopy bulk density (CBD) average for low, mid and high elevations classes. Different lower case letters (a, b, c) indicate significant differences between elevation classes ( $\alpha = 0.05$ ).

Elevation	Ht (m)	CBH (m)	DBH			
			(cm)	CL (m)	CFL ( $\text{kg m}^{-2}$ )	CBD ( $\text{kg m}^{-3}$ )
Low	12.9 <sup>a</sup>	0.9 <sup>a</sup>	37.3 <sup>a</sup>	12.0 <sup>a</sup>	0.36 <sup>a</sup>	0.03 <sup>a</sup>
Mid	12.0 <sup>a</sup>	0.9 <sup>a</sup>	54.3 <sup>b</sup>	11.0 <sup>a</sup>	1.12 <sup>b</sup>	0.09 <sup>a</sup>
High	7.5 <sup>b</sup>	0.6 <sup>b</sup>	74.6 <sup>c</sup>	6.9 <sup>b</sup>	1.84 <sup>b</sup>	0.22 <sup>b</sup>

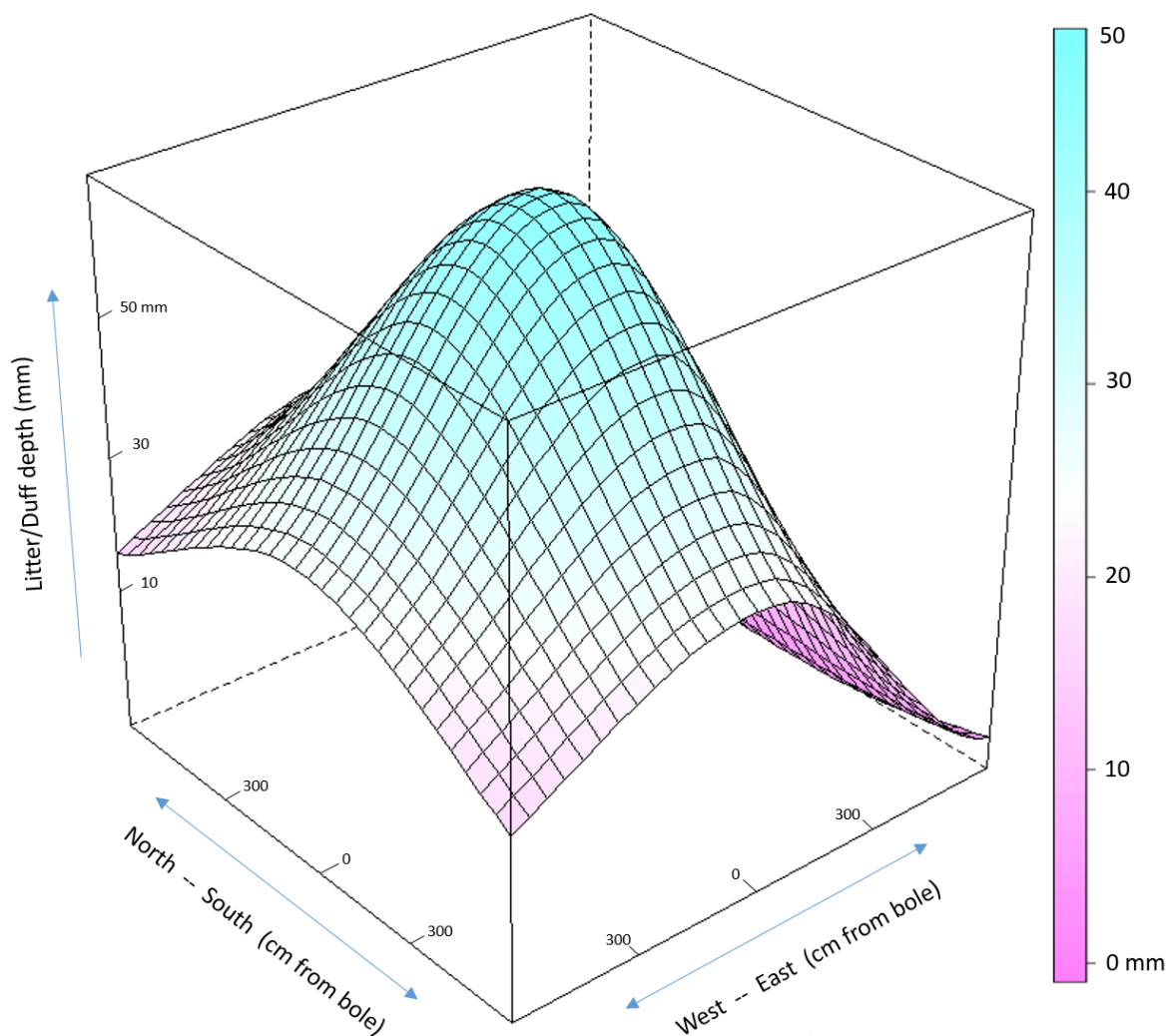
Foliar moisture content (FMC) at the three elevations sampled also significantly decreased at the upper elevation site (ANOVA with  $p < 0.001$ ) while the mid and low sites were not significantly different (Fig. 12). FMC varied significantly by month. September had the highest FMC and July the lowest (Fig. 12) with values likely influenced by monsoonal precipitation events.



**Fig. 12** Live foliar moisture content (FMC) collected from Great Basin bristlecone pines at low, mid, and high elevations.

#### *4.4. Individual tree fuel loading*

Measurements of litter and duff in the four cardinal directions (NSEW) directly beneath GBBP trees showed higher litter and duff fuel loads near the bole of the tree (Fig. 7, Fig. S4, and Fig. 13. Fitted curve of regression model of combined litter and duff around the bole of a tree. Fitted curves in each cardinal direction correspond to logistic regressions with a negative binomial link.). A negative binomial model was fit to the combined litter and duff for each cardinal direction (Fig. S4). The fitted curve for each cardinal direction was draped on a surface (mm of litter and duff) around the tree to visualize fuel loads underneath an individual tree (Fig. 13). While there might not be sufficient fuels between individual trees to carry a surface fire, nearly each individual tree had a pocket of litter and duff directly beneath it.



**Fig. 13.** Fitted curve of regression model of combined litter and duff around the bole of a tree. Fitted curves in each cardinal direction correspond to logistic regressions with a negative binomial link. (Vertical exaggeration = 300x)

## 6. Discussion

The sky islands of the Great Basin are predicted to experience a 2-4 °C increase in March-April mean temperature, a 60-100% decrease in mean April snow water equivalent (SWE), yet possibly experience a 10-20% increase in October-April precipitation by the end of the 21st century (Scalzitti et al., 2016). While there is uncertainty in how climate change will affect mountain regions of the Great Basin,

extensive research has modelled probable affects. Salzer et al. (2014) used tree ring chronologies to show that GBBP have a positive growth-response to increasing temperature at elevations 60–80 vertical meters below treeline in the White Mountains of California. Chronologies from 80 m and farther below treeline are sensitive to precipitation change (similar to lower elevation chronologies) and do not correlate strongly with temperature (Salzer et al., 2014). Using dynamical downscaling and applying a mean monthly perturbation to boundary conditions to simulate future climate, the role of temperature and precipitation in spring snowpack variability has been modeled throughout the western U.S. (Scalzitti et al. 2016, IAP-Climate Change Assessment). Assuming that GBBP treeline communities are temperature limited, it is reasonable to expect the stands to more closely approximate the conditions that are currently at the mid and low elevations (historically 2-4 °C warmer on average). Our research indicates that if climate warming changes fuel conditions, then the frequency of fire in GBBP systems at low and mid elevations could increase where stands are typically denser and surface fuel is greatest. While rare, wild fires such as The Carpenter 1 fire in southern Nevada (July, 2013; 36.25, -115.69) and the Phillips Fire in Great Basin National Park, (September, 2000; 38.90, -114.31) that started in lower elevation fuel types and moved through the crowns of GBBPs with the aid of extreme fire weather, could become more likely. The accumulation of fuels in lower elevation vegetation communities have proven to amplify the effects of fire in the high elevation/low fire return interval systems.

Warmer temperatures will likely increase the number of days in each fire season that FWD will be at its driest. FWD are fast drying fuels, which greatly affect ignition and spread of surface fires; CWD responds to changes in moisture and temperatures over

months and affect the latency of wildfires more than the rate of spread. FWD (1 hr, 10 hr, and 100 hr fuels) for all fuel sizes decreased with elevation in GBBP transects (Fig. 8), although this pattern did not hold for CWD (1000 hr fuels). Litter depth in GBBP sites ranged from 12 mm at the lower elevation sites to 5 mm at treeline sites while duff depth ranged from 10 mm to 3 mm, respectively. While the trend of decreasing surface fuels with elevation is consistent across most fuels types, there is considerable variation across the sample sites ( $R^2$  values ranged from 0.15 to 0.30). Disparities could be from the influence of other species, such as Engelmann spruce and limber pine, that contribute to stand structure, composition, and fuels. When surface fuels are contiguous, flames easily advance across the landscape, conversely, surface fires do not spread with discontinuous fuels, such as those in this study's high elevation sites. This confirms previous observations that fires in low-density stands near treeline were likely rare and small, due to the scarcity and discontinuity of fuels (Bailey, 1970). Litter and duff depths found in other Great Basin fuels plots ranged from mean depths of 20 mm and 6 mm, respectively, at the Clover Mountains dominated by ponderosa pine, to 12 and 3 mm, respectively, at Mount Irish dominated by piñon juniper (Cheek et al., 2012). Yet we measured litter fuel depths closer to 35 to 50 mm, which consider the high amounts of litter fuels that are directly beneath individual trees (Fig. S4 and Fig. 13). Litter fuel depths decrease to values closer to the FIA measurements at about 3 meters from any individual tree. Combined with patches of shrubs and prostrate spruce thickets, fire susceptibility may be higher than indicated by FIA measurements since FIA plot centers are usually not located directly beneath trees. If fire starts in low elevations and extreme fire weather exists, the unique patchy fuels found at mid and high elevations could make upper elevations more

susceptible to fire. This is not apparent from standard fuel models developed for lower elevation fuel types.

Drier fuels at all elevations increases fire hazard. Additionally, for GBBP, CFL and CBD increase at higher elevations (Table 6), resulting in denser crown fuels within individual trees. Crown fires likely are dependent upon weather, especially atmospheric stability and wind speed. Crown fire prediction models use canopy bulk density (CBD) as the index of canopy fuel characteristics to determine whether fire will initiate and spread either vertically or horizontally through a forest canopy. CBH is another important factor in predicting crown fires (Scott, 2006). CBH is less than 50 cm at the highest elevations of GBBP (Fig. 11. Great Basin bristlecone pine crown base height (CBH) (the distance from the ground to the first tree branches) versus elevation.), and live FMC (Fig. 12) decreased from 80-87% at low elevations to 71-78% at the highest elevations. This suggests that while the likelihood of a surface fire decreases with elevation, the potential for crown fire and spotting is still present. Thus, the greatest fire threat to GBBP growing near treeline is from fires ignited in the mixed conifer forests below and progressing as crown fires into pure GBBP stands during which threshold wind conditions and fuel moistures are exceeded. More research is needed to identify wind speed and fuel moisture threshold conditions that would sustain fire propagation.

In other Great Basin forest types, CBD at Mount Irish (37.64, -115.40) ranged from 0.04 to 0.18 kg m<sup>-3</sup> in juniper types, to 0.02 to 0.23 kg m<sup>-3</sup> at the Clover Mountains (37.40, -114.33) (Cheek et al., 2012), indicating higher severity potential crown fire behavior at low elevations. Crown fires normally occur because of steep topography, high wind speeds (Wolf, 2003), and canopy bulk density (CBD) of 0.05 kg m<sup>-3</sup> or more (van

Wagtendonk, 2006). While the samples of GBBP at the low elevations had relatively low CBD (0.03 to 0.09 kg m<sup>-3</sup>, Table 6), these trees are co-located with other tree species (piñon, juniper, and ponderosa pine) so that CBD and ICD sometimes support crown fire and high surface fuel loads. The composition and structure of the adjacent forests at the lower elevation margins of GBBP varied greatly and often have a large shrub component. A thorough inventory of these lower margins is necessary to predict the hazard to GBBP. GBBP pines at the highest elevations had a mean CBD of 0.22 kg m<sup>-3</sup> in the areas with the lowest CBH, while the ICD at these elevations becomes too large to carry a crown fire. While CBH and FMC decrease in GBBP at higher elevations (Fig. 11 and Fig. 12), the decreased stand density (Fig. 9), which likely reduces ICD (Jenkins, 2011), reduces the probability of a moving crown fire. The higher CBD values at high elevations may not be representative however, because many of these ancient trees become krumholtz (twisted and deformed) with only a portion of the tree remaining alive, which means that the allometric equation derived for whitebark pine may not be applicable to these trees. Fires that spread beyond individual trees are dependent on increasing fuel types and amounts from climate warming and introduction of lower elevation species.

For decades, fire suppression has contributed to forest stand-level effects such as: increases in woody fuel loading, canopy cover, vertical fuel distribution, canopy stratum, and fuel continuity, which in turn leads to increased fire intensity, severity, and size as fuels increase and become more connected. Interestingly however, fire suppression, which produced unnatural fuel accumulations in most locations in the American West, was not applied to high elevation pine forests where GBBP are located (Schoennagel et al., 2004). Regardless, quantifying GBBP fuels is important for forest management.



Large, severe fires have sizable economic implications when considering the costs of fire suppression, loss of valuable timber, poor air quality, soil erosion, as well as impacts on downstream water quality. Although stand replacing fires are rare, the more precise data and analysis presented here identifies areas that are the most susceptible to fire.

Management of GBBP stands might be novel or unprecedented, but fuels management could have large impacts on preventing stand replacing fire in these highly prized communities. How GBBP communities regenerate is also of great interest. Post-fire establishment may be favored in certain mixed conifer ecotones, lower-elevation limestone soils, and other sites that are marginally productive for other conifer species but relatively good for GBBPs.

Considering the recent ecological impacts of mountain pine beetle epidemics and blister rust in whitebark and limber pines, a major component of treeline in the northern Rocky Mountains, more attention to insect and disease epidemics in GBBP communities is needed. Recently, tree chemistry research showed that bark beetles are not attracted to GBBP (Gray et al., 2015). Tomback and Resler (2007) examined the cascading ecological effects of losing treeline whitebark pine and expected changes in landscape vegetation patterns in the context of climate change. They speculate that exotic pathogens could potentially confound predictions of treeline responses to global warming in many geographic regions and other communities (Tomback and Resler, 2007). Resistance of GBBP to blister rust is unknown, yet laboratory experiments suggest GBBP lack genetic resistance (Kinloch Jr and Dupper, 2002). More research is needed on these topics.

## 7. Management Implications

Overall, our knowledge of GBBP ecosystems is poorly understood. For example, GBBP longevity still remains a mystery, but improved understanding may be beneficial for creating appropriate adaptation strategies to manage this species with a changing climate. Historically, GBBP pine stands experienced low to high severity fires, and fuels structures changed considerably across elevational gradients. In low elevation, mixed species stands, fuels are often heavy and in close proximity to anthropogenic ignition sources. Yet at high elevations, GBBP typically grow on limestone outcroppings that provide little or no surface fuels to propagate a wildfire. In other words, stands are non-uniform so it is difficult to make blanket fire response predictions. In the absence of fire, stands are likely to exhibit gradual infilling by other local conifer, shade-tolerant tree species or invasive weed species with climate warming. Tree species that occur at the high-elevations and xeric margins provide ecological services that likely cannot be replaced. Climate change may manifest as warming temperatures with more days of extreme fire weather and reduced snowpack. Management in response to these threats to GBBP must balance a range of concerns and may include both the application and prevention of fire. How should fuels distribution be spread across the landscape? Should there be diversity at the stand level or in a mosaic of different patches and age distributions? How will we manage this with respect to climate change? Finally, how might management tools, including fire (or the lack thereof), be best used to encourage such conditions? Applied research is needed to gain insight into these questions. More research and improved fire models for patchy fuels are needed to predict and identify

appropriate fire management to protect valuable stands, promote regeneration, and diversify age classes.

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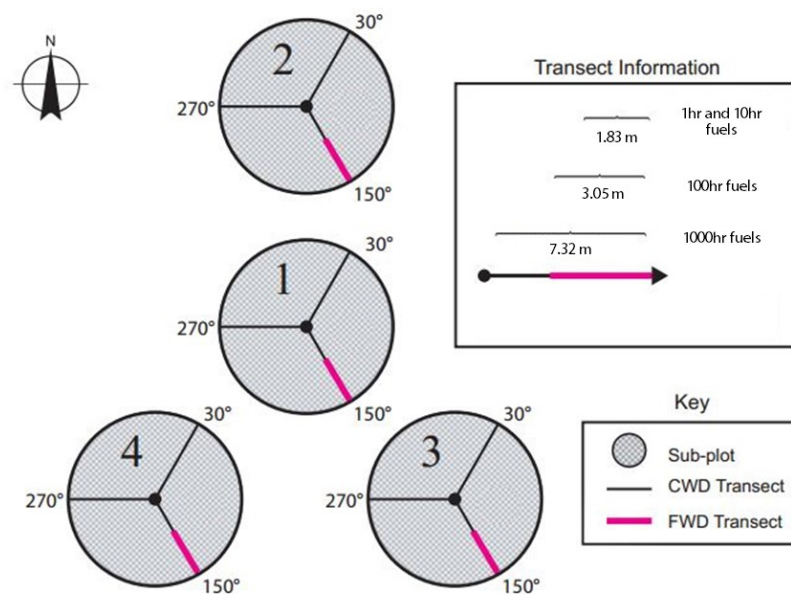
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## Supplemental Figures

A.

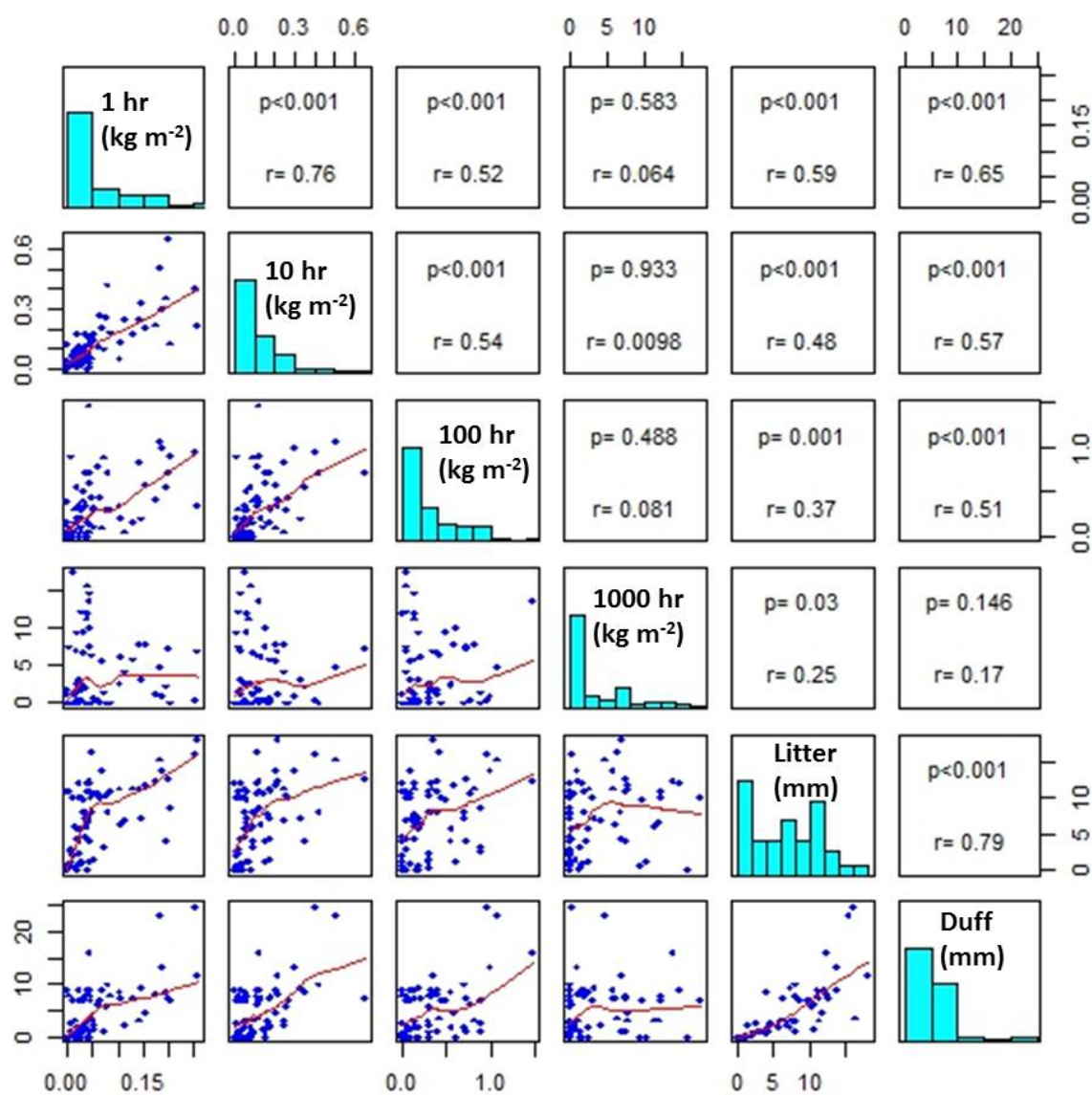


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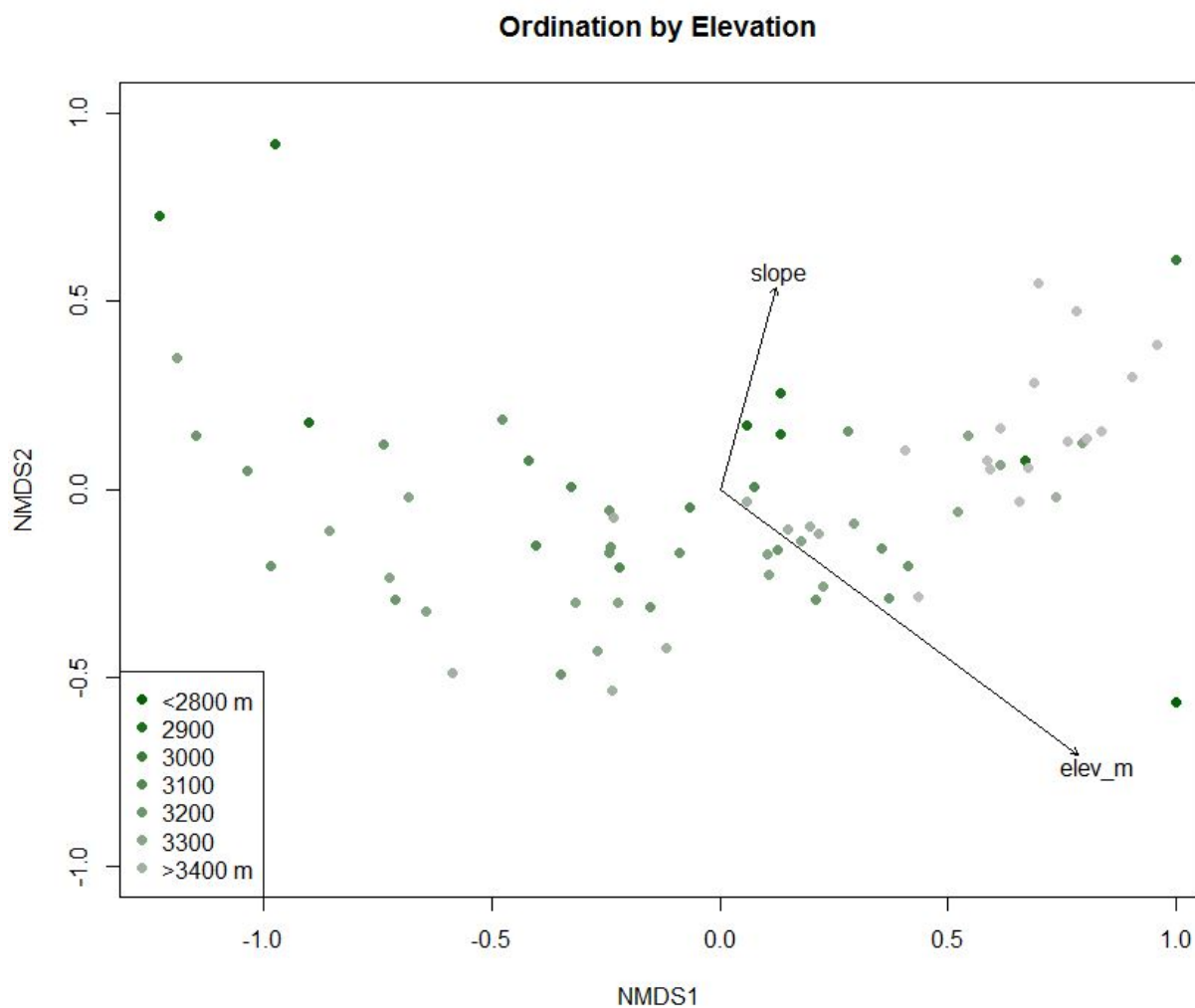


**Supplemental Fig. S1.** (A) Gradient transect of additional FIA protocol plots on Mt. Washington, NV. (B) FIA plot layout for stands structure and fuels sampling. Distance between sub-plot 1 and sub-plots 2, 3, & 4: 36.6 m at angles (degrees) 150, 210, and 270 respectively. Adapted from USFS Forest Inventory and Analysis field guide (2007).

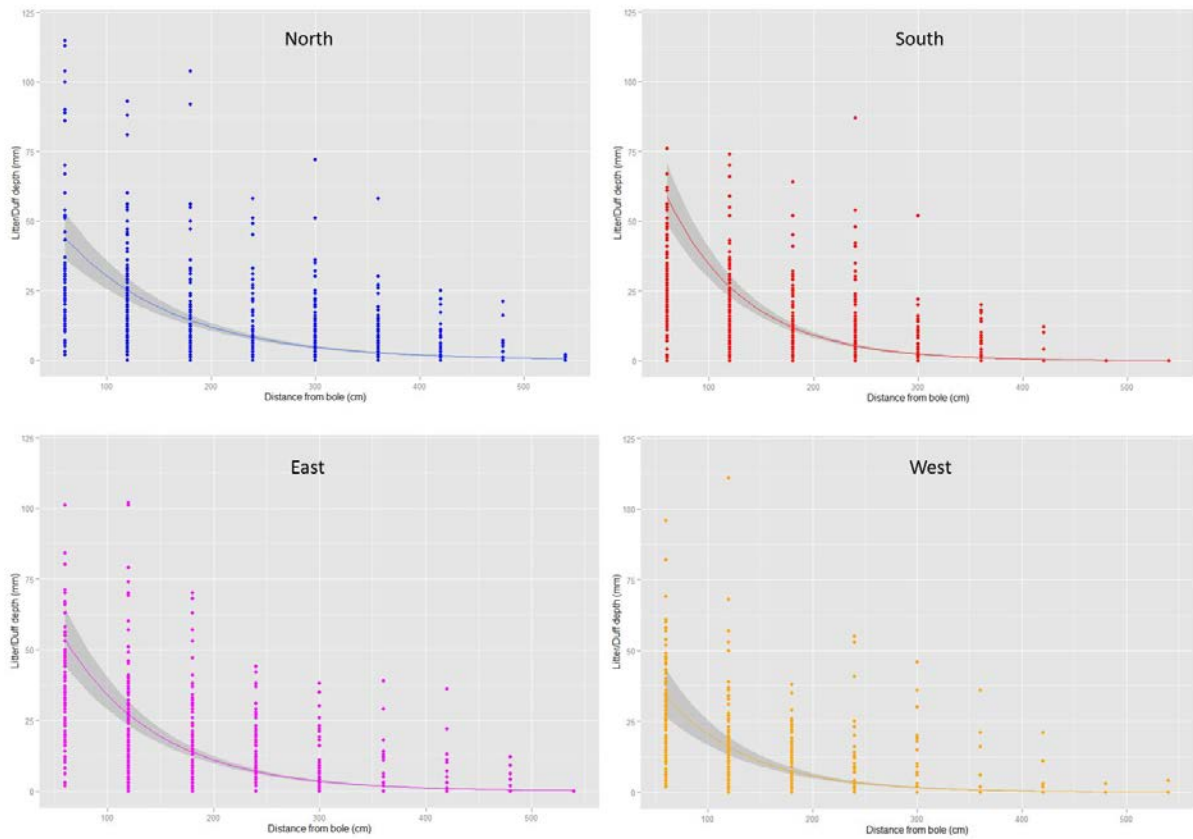




**Supplemental Fig. S2.** Correlation Matrix of coarse and fine woody debris (CWD and FWD), litter and duff. Fuel class along diagonal with histograms. Pearson's correlation coefficient (p value), correlation coefficient (r value) in upper right quadrants. Scatter plots with curve fitted using Lowess smoothing in lower left quadrant.



**Supplemental Fig. S3.** Non-linear Multidimensional Scaling (NMDS) plot of surface fuels components (fine woody debris (FWD), coarse woody debris (CWD), and litter and duff) in Great Basin bristlecone pine (*Pinus longaeva* Bailey) stands. Black vectors are environmental gradients that are significantly correlated ( $\alpha = 0.05$ ) to the fuels ordination. Points are plots along sampling transect, colors indicate elevation class, and points closer to each other are more similar in fuels characteristics.



**Supplemental Fig. S4.** Combined litter and duff depth (mm) from each cardinal transect sampled at 60cm intervals from the bole of the tree. Fitted curves correspond to logistic regressions with a negative binomial link. Gray lines around the curves are the 95% confidence interval.

## CHAPTER 4

GREAT BASIN BRISTLECONE PINE VOLATILES AS A CLIMATE CHANGE  
SIGNAL ACROSS ENVIRONMENTAL GRADIENTS**Abstract**

Alpine treeline species, like Great Basin bristlecone pine (GBBP) (*Pinus longaeva* Bailey), have received attention for their potential as indicators of climate change. Most studies have focused on climate-induced changes to treeline position, but climate effects on the physiology and stress of treeline plants remain poorly understood. Volatile organic compounds (VOCs) could provide insights into plant responses to climate change since the blends of VOCs released by plants exhibit variation in response to the environment, and can convey information about the status of the emitting plant. We collected and analyzed GBBP VOCs and within needle chemistry along elevational gradients (lower treeline, upper treeline, and midway in between) near the northern and southern geographic limits of GBBP. Random Forest analysis distinguished elevation classes using VOCs with 83% accuracy and identified the compounds most important for classification. Ordination revealed that temperature, heat load index, and relative humidity were each significantly correlated with VOCs. Within-needle chemistry provided less predictive value in classifying elevation class (68% accuracy) and was correlated only with heat load index. These findings suggest that GBBP VOCs are highly sensitive to the environment and could be used to assess and predict tree status and responses to environmental change. The potential effects of climate- and elevation-induced changes in GBBP chemistry on abiotic and biotic interactions are discussed.

## Introduction

Treeline species that live in harsh, high-elevation environments are particularly susceptible to climate change and can serve as early signals of change (Körner 1998, 2012). This is thought to be because trees growing near treeline are at or near their survival limits and so should be highly sensitive to environmental change and respond earlier than the rest of the forest (Smith et al. 2009, Körner 2012). Components of climate change such as temperature, elevated concentrations of CO<sub>2</sub>, and altered precipitation can each affect alpine treelines, with warming being the best studied (Grace et al. 2002, Smith et al. 2009, Körner 2012). For example, warming temperatures can alter alpine treelines by increasing tree mortality, promoting invasive plant establishment, changing forest fuels, plant community structure, and altering snowfall and melt patterns (Flannigan et al. 2000, Gibson et al. 2008, Balch et al. 2013). Warming temperatures can also increase risk of mortality to treeline species by stimulating abiotic and biotic disturbances such as mountain pine beetles (*Dendroctonus ponderosae* Hopkins), white pine blister rust (Tomback and Resler 2007), and altering natural fire regimes (Gibson et al. 2008, Gray and Jenkins 2017). To date, most studies that have examined climate effects on alpine treelines have focused on the advance or retreat of treeline position (Gehrig-Fasel et al. 2007, Paulsen and Körner 2014, Schibalski et al. 2014, Millar et al. 2015, Bruening et al. 2017). Climate effects on the physiology of treeline plants have received much less attention and remains largely unknown, despite the fact that understanding the impacts of environmental change on plant physiology could help predict how treelines will change.

Great Basin bristlecone pine (*Pinus longaeva* Bailey) (GBBP) is an alpine treeline, five-needle pine that has served as an important indicator of climate change (Körner 1998, Rochefort et al. 1994, Salzer et al. 2014, 2009). GBBPs are the oldest trees on earth, with some individuals exceeding 5,000 years old. This longevity has allowed GBBP to play an important role in climate change research due to their extremely long tree-ring chronologies (Cook et al. 1995, Cook and Peters 1997) with recent studies finding increased tree-ring growth at the highest elevations (Salzer et al. 2014, 2009). GBBP distribution is confined to the highest mountains (2700-3700m) of the Great Basin in the western United States, where global climate models predict a mean 2-4 °C increase in annual temperatures within the next several decades (Scalzitti et al. 2016, IAP-Climate Change Assessment) accompanied by a likely decrease in precipitation (Cook et al. 2010). The physiological responses of GBBP to warming temperatures and decreases in precipitation are largely unknown. Moreover, such warming could add stress by increasing wildfire activity (Westerling et al. 2006) in these habitats with historically sparse fuel conditions, in part by increasing fine woody surface fuels (Gray and Jenkins 2017).

Volatile organic compounds (VOCs) could be useful for detecting and measuring plant physiological responses to environmental change because the quantity and composition of VOCs emitted by a plant can be affected by the environment (Peñuelas and Staudt 2010, Dudareva et al. 2006, Jaeger et al. 2016). Plant VOCs have many known ecological roles, such as attracting pollinators (Burkle and Runyon 2016) and plant defense against herbivores, pathogens, and parasitic plants (De Moraes et al. 2001, Gray et al. 2015, Huang et al. 2012, Runyon et al. 2006). VOCs emitted by GBBP also

play important roles in defense, for example by repelling host-searching mountain pine beetles (Gray et al. 2015). Plant VOCs can also protect against certain abiotic stresses, including high temperatures and oxidative damage (Holopainen and Gershenzon 2010). In general, both biotic and abiotic stress increases VOC emissions from plants (Holopainen and Gershenzon 2010), and trees are known to emit VOCs in response to herbivory and changes in heat, light, precipitation, and season (Helmig et al. 2007, Trowbridge et al. 2014). Moreover, elevated temperatures typically increase VOC emissions (Tingey et al. 1980), and elevated CO<sub>2</sub> can increase emission of volatile terpenoids (Himanen et al. 2009, Yuan et al. 2009, O'Neill et al. 2010), which may become more common, especially at high elevation treeline (Peñuelas and Llusà 2003).

This study quantifies GBBP tree chemistry across elevational gradients near the southern and northern extent of GBBP's distribution as an approximation for future climate conditions. Elevation gradients are valuable surrogates for inferring broader climate change effects by providing variation in abiotic factors (Beier et al. 2012, Hodkinson 2005, Körner 2007). Moreover, because the elevation gradients at each latitude used in this study occurred over short distances (< 2.5 km), this minimized the confounding effects of biogeographical differences such as the community of plants, herbivores, and pathogens that are present (Hodkinson 2005). We address the following research questions in this paper:

- How does GBBP chemistry change with elevation, and is this correlated with air temperature, relative humidity (RH), and heat load index and thus to climate change?
- Do VOCs emitted from GBBP increase at lower elevations as a proxy for climate warming?

- Can we apply understanding of GBBP response to elevational gradients to interpret potential susceptibility to threats like fire or mountain pine beetles?

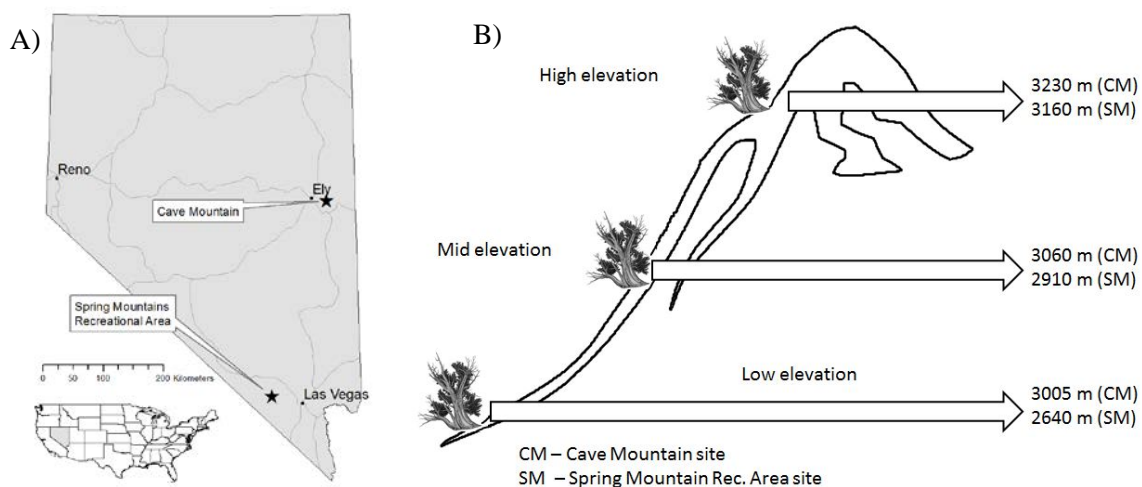
## Materials and Methods

### *Study Sites*

The Great Basin of western United States has an arid climate and basin and range topography, with numerous high mountain peaks (sky islands) separated by low intermountain valleys. In the Great Basin, upper treeline occurs at approximately 3,300 m and lower treeline at approximately 2,100 m (Thompson and Mead 1982), although this varies with latitude. Mountains and basins create steep environmental gradients, which greatly influence the composition and structure of vegetative communities (Peet 2000). For this study, we exploited these environmental gradients by sampling GBBP trees in two sky islands in Nevada near the northern and southern extremes of GBBP's geographic range. Sample transects were installed at Cave Mountain, NV (39.167, -114.616) and the Spring Mountains National Recreation Area, NV (36.293, -115.686) (Figure 14A). Three discrete elevation classes, "low" "mid" and "high", were sampled at each site. The low elevation plots were established at the lower extent of GBBP (3005 m at Cave Mtn, 2640 m at Spring Mtns), high elevation plots at upper treeline (3230 m at Cave Mtn, 3160 m at Spring Mtns), and mid elevation plots halfway between the two (3060 m at Cave Mtn, 2910 m at Spring Mtns) (Figure 14B). For each elevation class, all trees sampled were within  $\pm 10$  m elevation. Other tree species occurring at the plots (not all species occurred at all plots) included limber pine (*Pinus flexilis* James), Engelmann spruce (*Picea engelmannii* Parry), subalpine fir (*Abies lasiocarpa* var. *latifolia* Nutt.),



aspen (*Populus tremuloides* Michx.), piñon pine (*Pinus edulis* Engelm.), juniper (*Juniperus spp.*), mountain mahogany (*Cercocarpus ledifolius*), ponderosa pine (*Pinus ponderosa* Douglas), and white fir (*Abies concolor*).



**Figure 14.** Location of Great Basin bristlecone pine study sites in Nevada (A) and elevational gradients for both study sites (B) that were established using lower extant of GBBP (Low), upper treeline (High), and halfway between the two (Mid).

At each site and elevation, four GBBP trees of similar size were haphazardly selected that showed no obvious signs of stress (e.g. herbivory, pathogen attack). Mean height of sampled trees was  $13.5 \pm 0.8$  m, and mean diameter at breast height (dbh) was  $74.5 \pm 7.8$  cm ( $n = 24$ ). Trees were sampled once each month from July to September in 2013, at each site, it took field crews two days to sample trees from the three elevations. VOCs were sampled three times daily (1000, 1200, and 1400) in the first week of July, August, and September (total of 18 sample periods per month), needles for within-needle terpene concentrations were collected from each tree at the end of the day (1400). The July samples from the Spring Mtns were omitted from analysis because sampling were affected by the nearby Carpenter 1 fire (July, 2013, 36.25, -115.69, ~4 km away) (total

VOC collections: Cave Mtn = 108, Spring Mtns = 72). Other environmental variables measured at time of sampling were temperature (°C), relative humidity (RH in %), and topographic heat load index (HeatI). Heat load index (HeatI), or the potential direct radiation at a site, is not symmetrical, as a slope with afternoon sun will be warmer than an equivalent slope with morning sun. Heat load index was calculated using the equation:  $\text{HeatI} = 1 - \cos(\theta - 45) / 2$ , where  $\theta$  = aspect in degrees east of north, as an approximation of heat, rescaling aspect to a scale of zero to one, zero being the coolest slope, and one being having to most direct radiation (McCune and Keon 2002). The only environmental variables measured for within needle compounds were heat load index and elevation, as needles were collected once at the end of a sampling day.

#### *Collection and analysis of VOCs and within-needle chemistry*

Volatile emissions were collected by enclosing 50 cm of the apical end of one randomly selected branch with clear Teflon bags (50 cm wide x 75 cm deep, American Durafilm Co., Holliston, MA, USA). The apical branches were approximately 1.5 m above the forest floor, following the methods of Page, Jenkins, and Runyon (2012) and Gray et al. (2015). The same branch on each tree was marked with flagging and was used for all VOC sampling periods. Air was pulled from a side port ( $0.5 \text{ l min}^{-1}$ ) of the Teflon bags through volatile traps containing 30 mg of the porous polymer adsorbent HayeSep-Q (Restek, Bellefonte, PA, USA) using portable volatile collection systems comprising automated vacuum pumps (Volatile Assay Systems, Rensselaer, NY, USA). VOCs were collected for 30 min and Teflon bags were removed between sample times. VOCs were eluted from traps with 200  $\mu\text{l}$  of dichloromethane, and 1,000 ng of *n*-nonyl acetate was

added as the internal standard. After the last sampling period in September, the branches sampled for VOCs were clipped and needles removed and weighed.

To measure within-needle chemistry, approximately 20 g of needles from randomly selected branches (1 to 2 m above the forest floor) on the same trees were removed at the end each sampling date and stored on ice in a cooler (for 2-3 days) and then in a freezer at  $-80^{\circ}\text{C}$  until processed. Terpenoids were extracted from GBBP foliage following methods of Ormeno et al. (2009) and Page et al. (2014). For each sample, needles were thoroughly mixed and 2 g randomly selected and ground into a fine powder in liquid nitrogen using a mortar and pestle. Approximately 0.1 g of powdered needles were transferred into 2-ml FastPrep tubes (MP Biomedicals, Solon, OH), and 1.5 ml of cyclohexane was added and sonicated at room temperature for 20 min. Vials were then centrifuged at 13,000 g for 1 min and 200  $\mu\text{l}$  of cyclohexane (top layer) was transferred to a gas chromatograph vial and 1000 ng of *n*-nonyl acetate was added as the internal standard.

VOC and needle samples were analyzed using an Agilent 7890A gas chromatograph (GC) coupled with a 5975C mass spectrometer (MS) and separated on an HP-1 ms (30 m x 0.25 mm inside diameter, 0.25  $\mu\text{m}$  film thickness) column, helium was used as the carrier gas. The GC oven was maintained at  $35^{\circ}\text{C}$  for 3 min and then increased by  $5^{\circ}\text{C min}^{-1}$  to  $200^{\circ}\text{C}$ , then  $25^{\circ}\text{C min}^{-1}$  to  $250^{\circ}\text{C}$ . Quantifications were made relative to the internal standard using ChemStation software (Agilent Technologies, Wilmington, DE, USA). Identification of compounds were made using the NIST 08 Mass Spectral Search Program (National Institute of Standards and Technology, Gaithersburg, MD, USA) and confirmed by comparing retention times and mass spectra with

commercial standards, when possible. Remaining unidentified compounds were labeled as unidentified monoterpenoids (MT), unidentified sesquiterpenoids (ST), unidentified benzenoids (B), or unidentified green leaf volatiles (GLVs). VOC emission rates were corrected for needle weight (ng/hour/gram) as were within-needle chemical concentrations which are reported on a fresh needle weight basis ( $\mu\text{g}/\text{gram}$ ).

### **Statistical analyses**

#### *Analysis of variance (ANOVA)*

Statistical analyses for VOCs and within-needle compounds were performed using the non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) (Kruskal and Wallis 1952) to identify compounds with significant ( $P \leq 0.05$ ) differences among elevations, and to test whether samples originate from the same distribution. To identify which elevation classes had significant differences in compounds, pairwise differences between all sampling periods were square root or log-transformed to normalize data and stabilize variance, and assessed with a Tukey's HSD means test (Sokal and Rohlf 1995) and calculated using the *Stats* package in the R v.3.3.1 statistical software (R Development Core Team 2016).

#### *NMDS ordination*

To characterize VOC dissimilarity along environmental gradients, a non-metric multidimensional scaling (NMDS) ordination based on a matrix of Euclidean dissimilarities (Dixon 2003, Kenkel and Orłóci 1986) was calculated on the rank order proportion of VOCs. Ordination is the ordering of sample units along an axis, multiple

axes, or a gradient (McCune et al. 2002), and is most often used in ecology for gradient analysis and is used to select the most important factors, separate patterns, and reveal unforeseen patterns and processes (McCune et al. 2002). The goal of NMDS is to collapse information from multiple dimensions into just a few, so that they can be visualized and interpreted. The iterative NMDS procedure defines position of variables in multidimensional space, constructs an initial configuration in 2-dimensions, regresses distances in the initial configuration against the observed distances, determines the stress, or the disagreement between 2-D configuration and predicted values from the regression, and if stress is high, repositions the points in 2 dimensions in the direction of decreasing stress. The scatter of points representing samples in NMDS ordination space is iteratively allowed to evolve until it resembles the observed dissimilarity matrix as closely as possible. This method is non-metric because stress, the measure of closeness of fit, is based on the ranking of dissimilarity values rather than actual values of dissimilarity. The NMDS was based on a similarity matrix using the Bray–Curtis index (Clarke 1993) running with 600 iterations to permit the NMDS algorithm to develop an ordination with minimal stress. NMDS ordinations were completed using the *VEGAN* package (Dixon 2003) in R v.3.3.1 statistical software (R Development Core Team 2016). The ordination space was then visualized by overlaying environmental variables with the ordination to specifically address how the compounds respond to spatial variation in the natural environment.

### *Random Forest*

We used the Random Forest classification algorithm (Breiman 2001) to investigate whether VOCs hold value for classifying elevation (low, mid, and high).

Random Forest is a machine-learning algorithm that assigns samples to predefined groups and combines a consensus of multiple classification trees in numerous iterations and estimates the importance of each compound (Breiman 2001). Constructing multiple classification trees using hundreds of bootstrapped training sets and averaging the resulting predictions significantly reduces the variance and increases prediction accuracy compared with a single tree. Additionally, when the number of variables is very high (in this case 42 compounds), applying only a randomly selected subset of variables for each model is computationally efficient. This statistical method has been applied in other ecological studies to classify VOC samples to reduce noise and correctly identify volatile signatures of plants (Jaeger et al. 2016, Ranganathan and Borges 2010). Two Random Forest trials were carried out to classify elevation based on VOCs and within needle chemistry. These analyses used randomForest package v4.6-12 (Liaw and Wiener 2002) in R v.3.3.1 statistical software (R Development Core Team 2016) utilizing the optional measure of importance of predictor variables, and proximity, a measure of the internal structure of the data used to detect outliers, with number of trees set at 1000, and all other parameters were set as the defaults. Random Forest returns a confusion matrix that summarizes the accuracy of the classification as well as the variable importance. The importance of each VOC for classification was ranked using mean decrease in accuracy (MDA) which measures the accuracy in which the compound can be used to partition the data variables. The variables predicted to be important in the model help us to understand what variables are driving the differences in chemical signatures at different elevations. Random Forest has two measures of variable importance. The first is based on mean squared error (MSE) and relates to the prediction accuracy of the out-of-bag portion of

the data. The difference between the two MSEs are then averaged over all trees. The second measure describes variable importance based on the Gini impurity index, which is based on overfitted models (Breiman 2001).

Finally, regression coefficients of several ratios of compounds of interest were calculated using generalized linear models (GLM) relating the compound ratios to temperature gradients. Regression coefficients of flammable within needle compounds were calculated for elevational sites and heat load index. All statistics were completed using R v.3.3.1 statistical software (R Development Core Team 2016).

## **Results**

### *GBBP VOCs*

Gas chromatography–mass spectrometry (GC-MS) analysis identified 42 volatile compounds emitted by GBBP trees (Table 7). The majority of compounds were monoterpenoids (29 compounds, ca. 99% of total VOC emissions) followed by sesquiterpenoids (10 compounds) and benzenoids (3 compounds) (Table 7). These 42 compounds were emitted by all trees across all sample dates and elevations. However, volatile emissions varied quantitatively between study sites and across elevations for total VOCs and for some individual compounds (Table 7). VOCs increased with decreasing elevation for several compounds (highlighted in bold in Table 7) and this trend was common among the sesquiterpenoids. Amounts emitted for every sesquiterpenoid compound decreased with increasing elevation for at least one of the sites. Bornyl acetate was the only monoterpene to exhibit this inverse elevational trend at both study sites,

while three unidentified sesquiterpenoids (ST1, ST2, and ST4) showed this same elevational trend at both sites.

#### *Within-Needle Terpenoid Concentrations*

In total, 78 compounds were identified using GC-MS, the majority of which were monoterpenoids (46 compounds, ca. 91% of total terpene concentrations) and sesquiterpenoids (27 compounds, ca. 7.5% of total terpene concentrations). Overall, within-needle concentrations varied more by site than by elevation. Mean total within-needle terpene concentrations averaged across all sample periods were not significantly different among elevation classes, but the concentrations were significantly greater ( $P < 0.05$ ) at the Spring Mtns site in the south than the northern Cave Mtn site. Total within-needle terpene concentrations had high variability among elevation classes and study sites. As with emitted VOCs, some within-needle terpene compounds showed trends along elevational gradients with concentrations increasing with decreasing elevation (Table 8). Bornyl acetate again exhibited decreases in concentrations with increase in elevation at both sites, along with two unidentified monoterpenes (MT8 and MT). Two unidentified sesquiterpenes (ST2 and ST3) were correlated with elevational gradients at both sites, however, these compounds decreased with increasing elevation at the Spring Mtns site, and increased with elevation at Cave Mtn site.



**Table 7.** Volatile organic compounds (VOCs) emitted (ng/hour/gram) with standard error (SE) by foliage of Great Basin bristlecone pines at Spring Mountains and Cave Mountain, NV for low, middle and high elevations. VOC amounts are averaged across all sample periods (monthly for July-September 2013, VOCs collected daily at 1000, 1200, 1400 hr). Different lower case letters (a, b) indicate significant differences for a compound among elevation classes at a site ( $\alpha = 0.05$ ). Compound amounts that are inversely related to elevation are highlighted in bold. Named compounds were identified by comparing retention time and mass spectra with authentic standard. MT = unidentified monoterpene; ST = unidentified sesquiterpene; B = unidentified benzenoid.

$\mu$ Temp(°C)	Spring Mountains ( <i>n</i> = 72)						Cave Mountain ( <i>n</i> = 108)											
	Elevation		Middle		High		Elevation		Middle		High							
	Low						Low											
Compound	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE						
	(16.6 ± 0.4)		(13.1 ± 0.3)		(11.9 ± 0.4)		(19.3 ± 0.6)		(16.2 ± 0.8)		(15.0 ± 0.9)							
<b>Monoterpenes</b>																		
tricyclene	3.438	0.434	a	8.197	0.822	b	2.514	0.327	a	3.122	0.503	-	2.554	0.328	3.448	0.211		
MT 1	1.393	0.201	a	3.176	0.499	b	0.807	0.117	a	1.869	0.253	ab	1.211	0.180	b	1.985	0.183	a
MT 2	4.689	1.011	ab	13.873	4.859	a	2.693	0.342	b	6.047	0.987	a	3.013	0.421	b	4.216	0.567	ab
$\alpha$ -pinene	364.540	54.797	-	510.40	56.321	-	305.039	85.847	-	289.494	45.248	-	193.565	26.56	-	291.703	27.068	-
camphene	10.270	1.416	a	22.052	1.886	b	7.475	1.135	a	9.646	1.813	-	7.191	1.105	-	8.990	0.720	-
$\beta$ -pinene	32.035	5.504	a	68.046	5.613	b	29.251	4.349	a	57.077	19.645	-	29.816	4.712	-	37.948	3.180	-
$\beta$ -myrcene	6.319	1.242	a	7.472	0.790	a	2.895	0.455	b	8.066	1.367	a	4.733	0.814	b	5.820	0.542	ab
3-carene	0.216	0.030	a	0.590	0.109	b	0.135	0.017	a	0.546	0.083	-	0.353	0.061	-	0.411	0.052	-
<i>p</i> -cymene	0.999	0.174	a	3.442	0.501	b	0.705	0.103	a	1.964	0.382	a	1.036	0.238	b	1.118	0.115	ab
$\beta$ -phellandrene	28.988	4.907	a	58.668	8.052	b	19.245	2.678	a	53.058	8.506	-	35.999	6.418	-	40.028	3.326	-
limonene	4.245	0.698	a	6.711	0.665	b	2.482	0.441	a	5.082	0.723	-	3.428	0.529	-	4.440	0.422	-
<i>E</i> - $\beta$ -ocimene	0.301	0.059	a	0.525	0.097	b	0.174	0.033	a	<b>0.445</b>	<b>0.104</b>	<b>a</b>	<b>0.257</b>	<b>0.058</b>	<b>ab</b>	<b>0.154</b>	<b>0.022</b>	<b>b</b>
$\gamma$ -terpinene	0.433	0.118	a	0.505	0.068	a	0.131	0.018	b	<b>0.883</b>	<b>0.230</b>	<b>a</b>	<b>0.214</b>	<b>0.068</b>	<b>b</b>	<b>0.191</b>	<b>0.028</b>	<b>b</b>
terpinolene	1.829	0.285	a	3.946	0.561	b	1.211	0.238	a	2.658	0.565	a	1.300	0.296	b	1.609	0.186	ab
linalool	0.097	0.042	-	0.138	0.042	-	0.026	0.012	-	<b>0.461</b>	<b>0.125</b>	<b>a</b>	<b>0.094</b>	<b>0.053</b>	<b>b</b>	<b>0.036</b>	<b>0.008</b>	<b>b</b>
MT 3	0.210	0.046	a	0.413	0.062	b	0.139	0.035	a	0.300	0.080	-	0.233	0.067	-	0.246	0.053	-
MT 4	0.376	0.070	a	1.010	0.175	b	0.216	0.037	a	0.958	0.246	-	0.563	0.125	-	0.626	0.118	-
camphor	0.388	0.083	ab	0.517	0.092	a	0.164	0.033	b	0.155	0.023	-	0.185	0.024	-	0.218	0.024	-
MT 5	0.245	0.054	a	0.468	0.078	b	0.095	0.021	a	0.419	0.077	-	0.240	0.064	-	0.274	0.050	-
geranyl acetate	0.129	0.024	a	1.300	0.209	b	0.271	0.070	a	0.144	0.019	-	0.112	0.015	-	0.113	0.012	-
bornyl acetate	<b>1.219</b>	<b>0.235</b>	<b>a</b>	<b>1.123</b>	<b>0.151</b>	<b>a</b>	<b>0.421</b>	<b>0.105</b>	<b>b</b>	<b>2.135</b>	<b>0.857</b>	<b>-</b>	<b>1.078</b>	<b>0.362</b>	<b>-</b>	<b>0.597</b>	<b>0.112</b>	<b>-</b>
$\alpha$ -phellandrene	0.249	0.030	a	0.580	0.047	b	0.178	0.019	a	0.827	0.198	a	0.290	0.069	b	0.310	0.020	b
$\alpha$ -terpinene	0.121	0.024	a	0.259	0.038	b	0.055	0.010	a	<b>0.301</b>	<b>0.094</b>	<b>-</b>	<b>0.095</b>	<b>0.027</b>	<b>-</b>	<b>0.083</b>	<b>0.009</b>	<b>-</b>

Table 7 continued.

MT 6	<b>0.171</b>	<b>0.074</b>	a	<b>0.058</b>	<b>0.012</b>	ab	<b>0.016</b>	<b>0.006</b>	b	0.053	0.015	-	0.035	0.007	-	0.046	0.010	-
MT 7	<b>0.302</b>	<b>0.110</b>	a	<b>0.217</b>	<b>0.043</b>	ab	<b>0.046</b>	<b>0.014</b>	b	0.184	0.038	a	0.090	0.020	b	0.101	0.018	ab
MT 8	<b>1.894</b>	<b>0.886</b>	a	<b>0.106</b>	<b>0.026</b>	b	<b>0.062</b>	<b>0.025</b>	b	0.086	0.030	-	0.024	0.004	-	0.075	0.023	-
verbenone	0.157	0.029	-	0.258	0.057	-	0.103	0.027	-	0.151	0.039	-	0.136	0.034	-	0.150	0.030	-
MT 9	0.023	0.005	-	0.044	0.012	-	0.018	0.005	-	0.044	0.011	-	0.040	0.011	-	0.047	0.013	-
MT 10	<b>0.743</b>	<b>0.299</b>	a	<b>0.177</b>	<b>0.039</b>	a	<b>0.086</b>	<b>0.025</b>	b	0.277	0.092	-	0.081	0.019	-	0.110	0.023	-

**Sesquiterpenes**

<i>E</i> - $\beta$ -farnesene	0.317	0.056	-	1.279	0.311	-	0.279	0.056	-	<b>0.854</b>	<b>0.131</b>	a	<b>0.512</b>	<b>0.108</b>	ab	<b>0.461</b>	<b>0.083</b>	b
$\alpha$ -farnesene	0.311	0.060	-	0.702	0.188	-	0.229	0.065	-	<b>1.860</b>	<b>0.408</b>	a	<b>0.630</b>	<b>0.140</b>	b	<b>0.262</b>	<b>0.060</b>	b
caryophyllene oxide	0.024	0.004	-	0.026	0.003	-	0.017	0.006	-	<b>0.083</b>	<b>0.018</b>	a	<b>0.028</b>	<b>0.007</b>	b	<b>0.016</b>	<b>0.003</b>	b

Table 7 continued

ST 1	<b>1.947</b>	<b>0.361</b>	-	<b>1.636</b>	<b>0.309</b>	-	<b>1.082</b>	<b>0.273</b>	-	<b>1.721</b>	<b>0.444</b>	-	<b>1.555</b>	<b>0.727</b>	-	<b>1.359</b>	<b>0.290</b>	-
ST 2	<b>0.050</b>	<b>0.012</b>	a	<b>0.037</b>	<b>0.006</b>	ab	<b>0.018</b>	<b>0.004</b>	b	<b>0.093</b>	<b>0.019</b>	-	<b>0.064</b>	<b>0.023</b>	-	<b>0.057</b>	<b>0.011</b>	-
ST 3	<b>0.054</b>	<b>0.017</b>	-	<b>0.044</b>	<b>0.018</b>	-	<b>0.019</b>	<b>0.011</b>	-	0.029	0.007	-	0.037	0.014	-	0.029	0.006	-
ST 4	<b>0.116</b>	<b>0.026</b>	-	<b>0.073</b>	<b>0.023</b>	-	<b>0.051</b>	<b>0.015</b>	-	<b>0.109</b>	<b>0.031</b>	-	<b>0.095</b>	<b>0.049</b>	-	<b>0.076</b>	<b>0.021</b>	-
ST 5	0.049	0.013	a	0.129	0.018	b	0.026	0.006	a	<b>0.080</b>	<b>0.015</b>	-	<b>0.060</b>	<b>0.023</b>	-	<b>0.053</b>	<b>0.010</b>	-
ST 6	0.013	0.004	-	0.017	0.003	-	0.008	0.002	-	<b>0.046</b>	<b>0.014</b>	-	<b>0.022</b>	<b>0.006</b>	-	<b>0.015</b>	<b>0.004</b>	-
ST 7	0.046	0.016	-	0.024	0.005	-	0.212	0.182	-	<b>0.365</b>	<b>0.135</b>	a	<b>0.026</b>	<b>0.007</b>	b	<b>0.026</b>	<b>0.006</b>	b

**Benzenoid compounds**

methyl salicylate	0.198	0.041	ab	0.265	0.028	a	0.139	0.026	b	<b>0.475</b>	<b>0.065</b>	-	<b>0.403</b>	<b>0.111</b>	-	<b>0.380</b>	<b>0.061</b>	-
B1	<b>0.620</b>	<b>0.175</b>	-	<b>0.502</b>	<b>0.082</b>	-	<b>0.322</b>	<b>0.093</b>	-	1.899	0.474	-	1.928	0.734	-	0.792	0.124	-
B2	0.474	0.066	a	2.715	0.401	b	0.627	0.152	a	0.389	0.052	-	0.322	0.040	-	0.351	0.029	-

<b>Total volatiles</b>	470.24	73.738		721.7	83.320		379.68	97.43		454.45	84.16		293.64	44.6		408.97	37.85	-
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**Table 8.** Within-needle terpenoid concentrations ( $\mu\text{g/g}$ ) with standard error (SE) of Great Basin bristlecone pines at Spring Mountains and Cave Mountain, NV for low, middle and high elevations, averaged across sample periods (monthly for July-September 2013). Different lower case letters (a, b) indicate significant differences for a compound among elevation classes at a site ( $\alpha = 0.05$ ). Compound amounts that are inversely related to elevation are highlighted in bold. MT = unidentified monoterpene; ST = unidentified sesquiterpene; GLV = green leaf volatiles.

Compound	Spring Mountains ( $n = 18$ )						Cave Mountain ( $n = 27$ )					
	Elevation		Middle		High		Elevation		Middle		High	
	Low						Low					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<b>Monoterpenes</b>												
tricyclene <sup>1</sup>	44.71	6.77 -	60.98	9.11 -	55.74	12.83 -	49.79	5.24 -	46.69	8.39 -	54.61	4.23 -
MT 1	0.51	0.31 -	0.94	0.49 -	0.10	0.02 -	0.21	0.03 -	0.27	0.05 -	0.29	0.07 -
MT 2	<b>0.38</b>	<b>0.28 a</b>	<b>0.20</b>	<b>0.12 a</b>	<b>0.03</b>	<b>0.01 b</b>	0.05	0.01 -	0.04	0.01 -	0.08	0.02 -
$\alpha$ -pinene <sup>1</sup>	1266.6	185.99 -	1475.1	133.54 -	1395.1	249.41 -	2199.5	194.55 -	2150.2	207.4 -	2819.8	322.75 -
camphene <sup>1</sup>	118.73	18.53 -	158.86	26.39 -	137.62	33.29 -	120.04	15.48 -	106.32	21.85 -	123.37	11.08 -
$\beta$ -pinene <sup>1</sup>	373.28	61.03 -	388.18	46.21 -	681.42	134.95 -	796.36	121.91 -	670.82	84.74 -	743.90	85.31 -
$\beta$ -myrcene <sup>1</sup>	108.77	18.68 -	106.49	14.88 -	123.13	20.36 -	225.05	30.28 -	183.04	24.15 -	232.60	44.08 -
3-Carene <sup>1</sup>	2.90	0.46 -	2.57	0.48 -	3.51	0.60 -	6.98	1.15 -	6.00	0.77 -	6.49	1.01 -
<i>p</i> -cymene <sup>1</sup>	2.89	0.37 -	4.53	0.93 -	2.91	0.55 -	4.25	0.33 -	3.29	0.31 -	4.87	0.74 -
$\beta$ -phellandrene <sup>1</sup>	844.18	136.45 -	766.29	165.75 -	993.48	155.04 -	1365.1	146.32 -	1485.3	127.0 -	1470.3	115.48 -
limonene <sup>1</sup>	37.65	5.32 -	39.96	6.13 -	45.54	7.20 -	61.14	6.81 -	62.95	7.84 -	66.49	6.09 -
( <i>E</i> )- $\beta$ -ocimene <sup>1</sup>	<b>0.94</b>	<b>0.63 -</b>	<b>0.73</b>	<b>0.36 -</b>	<b>0.27</b>	<b>0.05 -</b>	0.49	0.06 -	0.41	0.05 -	0.51	0.07 -
$\gamma$ -terpinene <sup>1</sup>	2.65	0.38 -	3.99	0.59 -	3.13	0.49 -	6.73	0.81 ab	3.65	0.53 a	8.24	1.78 b
$\alpha$ -terpineol <sup>1</sup>	<b>7.91</b>	<b>2.47 a</b>	<b>1.04</b>	<b>0.66 b</b>	<b>0.31</b>	<b>0.21 b</b>	1.50	0.88 -	0.19	0.06 -	1.01	0.39 -
linalyl acetate <sup>2</sup>	1.29	0.32 -	0.75	0.21 -	1.73	0.63 -	24.34	9.02 a	0.96	0.16 b	4.80	2.00 b
MT 3	0.35	0.31 -	0.39	0.21 -	0.07	0.03 -	0.09	0.02 -	0.19	0.10 -	0.92	0.81 -
MT 4	0.19	0.08 -	0.42	0.19 -	0.05	0.02 -	0.08	0.02 -	0.09	0.02 -	0.10	0.03 -
camphor <sup>1</sup>	0.23	0.15 -	0.26	0.17 -	0.04	0.02 -	0.27	0.17 -	0.08	0.02 -	0.13	0.06 -
MT 5	0.22	0.06 -	0.42	0.10 -	0.19	0.05 -	0.38	0.08 -	0.26	0.06 -	0.41	0.08 -
borneol <sup>1</sup>	2.27	0.66 -	3.16	1.33 -	2.64	0.77 -	3.80	0.91 -	4.71	1.58 -	5.07	1.09 -
bornyl acetate <sup>1</sup>	<b>194.42</b>	<b>46.53 -</b>	<b>193.14</b>	<b>57.03 -</b>	<b>116.06</b>	<b>27.47 -</b>	<b>214.76</b>	<b>48.40 -</b>	<b>170.89</b>	<b>57.16 -</b>	<b>129.68</b>	<b>21.40 -</b>
$\alpha$ -phellandrene <sup>1</sup>	9.00	1.63 -	8.12	1.72 -	10.89	1.73 -	25.40	4.68 -	21.00	3.18 -	21.65	3.96 -
$\alpha$ -terpinene <sup>1</sup>	0.90	0.10 -	1.31	0.16 -	1.12	0.16 -	2.45	0.37 ab	1.43	0.22 a	2.82	0.50 b
$\alpha$ -thujene <sup>1</sup>	12.23	1.10 -	19.39	6.51 -	15.99	4.05 -	31.24	4.93 -	18.75	4.01 -	42.46	10.87 -
sabinene <sup>1</sup>	15.61	4.24 -	22.77	3.86 -	26.82	4.02 -	35.31	8.41 -	28.46	4.60 -	37.99	6.29 -

Table 8 continued.

MT 8	<b>0.88</b>	<b>0.39 a</b>	<b>0.69</b>	<b>0.30 a</b>	<b>0.35</b>	<b>0.09 b</b>	<b>0.68</b>	<b>0.07 -</b>	<b>0.64</b>	<b>0.10 -</b>	<b>0.58</b>	<b>0.06 -</b>
verbenone <sup>1</sup>	3.04	1.93 -	7.37	4.92 -	0.55	0.17 -	0.74	0.15 -	1.04	0.22 -	1.29	0.38 -
MT 9	<b>4.32</b>	<b>3.91 -</b>	<b>2.96</b>	<b>1.52 -</b>	<b>0.13</b>	<b>0.03 -</b>	<b>4.12</b>	<b>1.53 a</b>	<b>0.76</b>	<b>0.22 b</b>	<b>0.53</b>	<b>0.16 b</b>
MT 10	0.53	0.25 -	1.01	0.49 -	0.09	0.02 -	0.20	0.05 -	0.25	0.12 -	0.22	0.08 -
<i>cis</i> -verbenol <sup>1</sup>	12.32	9.25 -	17.22	10.26 -	0.46	0.12 -	1.29	0.27 -	1.93	0.49 -	2.50	0.77 -
terpinyl acetate <sup>1</sup>	<b>132.36</b>	<b>38.08 -</b>	<b>49.57</b>	<b>25.50 -</b>	<b>45.12</b>	<b>24.52</b>	76.74	30.61 -	19.56	2.57 -	61.25	22.38 -
<i>L</i> -pinocarveol <sup>2</sup>	3.64	2.46 -	6.44	3.75 -	0.20	0.07 -	0.40	0.16 -	0.18	0.11 -	0.38	0.24 -
( <i>Z</i> )- $\beta$ -ocimene <sup>1</sup>	<b>0.62</b>	<b>0.32 -</b>	<b>0.50</b>	<b>0.22 -</b>	<b>0.22</b>	<b>0.03 -</b>	0.42	0.08 -	0.28	0.07 -	0.33	0.07 -
MT 11	2.81	2.17 -	3.48	2.08 -	0.10	0.03 -	0.35	0.10 -	0.51	0.15 -	0.56	0.17 -
MT 12	0.41	0.28 -	0.97	0.56 -	0.04	0.01 -	0.06	0.02 -	0.10	0.02 -	0.19	0.05 -
MT 13	1.46	0.25 -	1.78	0.32 -	2.40	0.44 -	3.47	0.42 -	3.39	0.34 -	4.10	0.57 -
MT 14	0.60	0.28 -	1.10	0.40 -	0.26	0.05 -	0.37	0.21 -	0.08	0.07 -	0.24	0.15 -
MT 15	0.21	0.14 -	0.34	0.19 -	0.04	0.02 -	0.01	0.00 -	0.04	0.02 -	0.02	0.01 -
MT 16	1.22	0.29 a	4.70	1.19 b	0.52	0.18 a	2.40	0.38 -	1.33	0.34 -	1.75	0.32 -
MT 18	<b>2.03</b>	<b>0.76 -</b>	<b>1.76</b>	<b>0.25 -</b>	<b>1.57</b>	<b>0.43 -</b>	2.41	0.57 -	1.78	0.34 -	2.58	0.44 -
MT 19	1.23	1.04 -	1.40	0.80 -	0.06	0.01 -	0.08	0.04 -	0.06	0.03 -	0.08	0.04 -
MT 20	0.93	0.46 a	1.51	0.54 a	0.22	0.03 b	0.43	0.06 -	0.57	0.11 -	0.52	0.10 -
MT 21	0.46	0.10 -	0.55	0.12 -	0.29	0.07 -	<b>0.52</b>	<b>0.05 -</b>	<b>0.48</b>	<b>0.03 -</b>	<b>0.39</b>	<b>0.03 -</b>
MT 22	3.02	0.65 -	4.84	1.36 -	4.39	0.80 -	7.37	1.88 -	5.68	1.24 -	9.06	2.09 -
MT 23	<b>0.52</b>	<b>0.38 -</b>	<b>0.49</b>	<b>0.23 -</b>	<b>0.14</b>	<b>0.04 -</b>	0.25	0.08 -	0.25	0.09 -	0.27	0.07 -
MT 24	11.28	3.43 -	10.25	3.37 -	14.07	3.85 -	31.80	8.21 -	19.58	2.89 -	20.62	3.41 -
<b>Sesquiterpenes</b>												
( <i>E</i> )- $\beta$ -farnesene <sup>1</sup>	5.02	0.60 a	12.90	2.05 b	10.30	2.25 b	14.43	1.39 -	13.55	2.56 -	15.55	3.30 -
longifolene <sup>2</sup>	<b>27.68</b>	<b>11.97 -</b>	<b>20.74</b>	<b>4.84 -</b>	<b>14.24</b>	<b>2.84 -</b>	3.74	0.82 a	8.85	2.95 a	89.07	30.11 b
$\beta$ -caryophyllene <sup>1</sup>	147.72	35.78 ab	157.99	25.56 a	62.48	19.31 b	100.26	19.51 -	169.11	26.05 -	194.09	37.36 -
$\alpha$ -humulene <sup>1</sup>	21.45	5.39 ab	23.41	4.05 a	8.19	2.60 b	13.76	3.14 -	23.59	3.99 -	28.63	6.10 -
$\alpha$ -bisabolol <sup>2</sup>	3.51	1.06 -	3.60	1.59 -	4.12	1.55 -	2.70	1.67 -	3.57	1.95 -	7.73	2.93 -
caryophyllene oxide	11.99	2.18 -	14.82	4.39 -	6.50	1.93 -	9.89	1.01 -	12.39	1.71 -	15.93	3.83 -
ST 1 <sup>4</sup>	0.92	0.32 -	1.40	0.35 -	0.54	0.13 -	0.44	0.08 a	0.51	0.11 a	2.88	0.93 b
ST 2	1.81	0.43 ab	2.38	0.59 a	0.68	0.19 b	2.27	0.41 -	2.75	0.57 -	2.11	0.42 -
ST 3	<b>1.21</b>	<b>0.41 -</b>	<b>1.16</b>	<b>0.34 -</b>	<b>0.77</b>	<b>0.15 -</b>	<b>0.55</b>	<b>0.06 a</b>	<b>0.70</b>	<b>0.09 a</b>	<b>3.45</b>	<b>1.19 b</b>
ST 4	<b>0.89</b>	<b>0.31 -</b>	<b>0.57</b>	<b>0.16 -</b>	<b>0.44</b>	<b>0.10 -</b>	<b>0.12</b>	<b>0.06 a</b>	<b>0.32</b>	<b>0.11 a</b>	<b>2.65</b>	<b>0.84 b</b>
ST 5	0.79	0.19 a	2.21	0.29 b	1.66	0.61 ab	3.98	0.66 -	2.30	0.52 -	3.36	0.76 -
ST 6	1.53	0.26 -	2.84	0.50 -	2.32	0.78 -	5.72	0.87 -	3.24	0.70 -	4.41	1.04 -
ST 7	0.14	0.07 -	0.43	0.10 -	0.32	0.11 -	0.70	0.16 -	0.29	0.14 -	0.77	0.25 -
ST 8	5.31	1.63 -	12.51	3.07 -	9.64	1.69 -	17.35	2.31 -	18.37	4.04 -	11.08	2.61 -
ST 9	2.83	0.48 -	4.41	1.43 -	1.38	0.29 -	2.05	0.39 -	2.80	0.42 -	3.23	0.52 -
ST 10	7.03	1.93 -	16.84	1.85 -	14.99	5.08 -	33.04	4.74 -	22.77	3.03 -	32.60	5.27 -

Table 8 continued.

ST 11	10.46	5.05 -	51.07	12.60 -	41.79	20.21 -	123.13	24.26 -	51.98	21.48 -	90.10	30.75 -
ST 12	3.16	1.37 a	16.93	5.39 b	8.91	3.85 ab	8.62	2.54 -	5.50	2.77 -	14.83	5.98 -
ST 13	2.49	0.82 a	9.56	1.88 b	7.88	2.67 ab	16.59	3.27 -	10.50	3.48 -	12.40	3.81 -
ST 14	<b>1.73</b>	<b>0.96 -</b>	<b>1.56</b>	<b>0.96 -</b>	<b>0.50</b>	<b>0.26 -</b>	3.18	1.59 -	1.58	1.15 -	2.44	2.37 -
ST 15	<b>0.90</b>	<b>0.24 -</b>	<b>0.55</b>	<b>0.31 -</b>	<b>0.22</b>	<b>0.09 -</b>	0.59	0.18 a	0.06	0.05 b	0.25	0.16 ab
ST 16	1.06	0.36 -	2.75	0.69 -	1.59	0.87 -	3.00	1.03 -	1.56	0.42 -	2.49	0.97 -
ST 17	0.90	0.34 a	2.65	0.55 b	1.09	0.30 ab	1.84	0.66 -	1.08	0.48 -	3.12	1.28 -
ST 18	0.13	0.07 -	3.95	2.89 -	0.16	0.08 -	0.94	0.58 -	0.23	0.17 -	0.49	0.39 -
ST 19	0.68	0.29 -	6.76	4.85 -	0.23	0.16 -	0.63	0.35 -	0.00	0.00 -	0.27	0.18 -
ST 20	0.77	0.28 -	57.32	43.01 -	1.48	0.29 -	6.94	2.32 -	2.27	0.81 -	3.36	1.00 -
ST 21	0.01	0.01 a	1.63	1.11 b	0.05	0.05 a	0.45	0.15 -	0.70	0.18 -	0.15	0.05 -
<b>Benzenoid</b>												
benzenoid 1	24.73	3.71 -	25.01	2.73 -	27.71	5.17 -	39.16	6.60 a	77.17	8.91 b	68.09	6.91 b
<b>GLVs</b>												
GLV 1	0.38	0.11 -	0.28	0.08 -	0.43	0.08 -	<b>0.84</b>	<b>0.21 -</b>	<b>0.76</b>	<b>0.12 -</b>	<b>0.66</b>	<b>0.18 -</b>
3-hexenal <sup>2</sup>	28.93	8.02 -	19.64	4.70 -	26.61	4.91 -	<b>40.81</b>	<b>8.15 -</b>	<b>34.46</b>	<b>4.70 -</b>	<b>26.34</b>	<b>6.48 -</b>
( <i>E</i> )-2-hexenal <sup>1</sup>	2.91	0.72 -	2.28	0.83 -	4.47	1.04 -	10.01	3.21 -	10.30	1.92 -	9.83	3.03 -
( <i>Z</i> )-3-hexenol <sup>1</sup>	2.69	0.52 -	1.43	0.44 -	1.57	0.42 -	2.05	0.48 -	4.35	0.77 -	3.28	0.95 -
<b>Total</b>	<b>3554.4</b>	<b>645.52 -</b>	<b>3860.4</b>	<b>669.65 -</b>	<b>3952.3</b>	<b>765.02 -</b>	<b>5779.2</b>	<b>738.61 -</b>	<b>5512.1</b>	<b>660.2 -</b>	<b>6541.7</b>	<b>833.67 -</b>

<sup>1</sup>Identity verified by comparing retention time and mass spectrum with authentic standard

<sup>2</sup>Identified using NIST 08 Mass Spectral Search Program

*Ordination of VOCs and within-needle compounds*

We used NMDS ordination to visualize elevational effects on VOCs composition (Figure 15). NMDS ordination of the VOC data had high linear fit ( $R^2 = 0.99$ ) and a low stress value (0.057). A stress of ca. 0.05 provides an excellent representation in reduced dimensions, while a stress of ca. 0.1 provides a good representation (Clarke 1993). The distances between points in Figure 15 are proportional to the dissimilarity between the blends of VOCs for each tree. While there is high variability among elevations, the first axis of the ordination plot (NMDS1) was highly correlated with temperature [positive] and humidity [negative], whereas the vertical axis (NMDS2) was highly correlated with heat load index [positive]. Note that  $\alpha$ -pinene,  $\beta$ -pinene, and camphene, the three most abundant VOCs, and limonene and 3-carene (compounds known to be important for tree defense (Gray et al. 2015, Raffa and Smalley 1995) are all clustered along the temperature gradient vector (Figure 15). Mean temperatures for Spring Mountains at low, mid, and high were 16.6, 13.1, and 11.9 °C respectively.

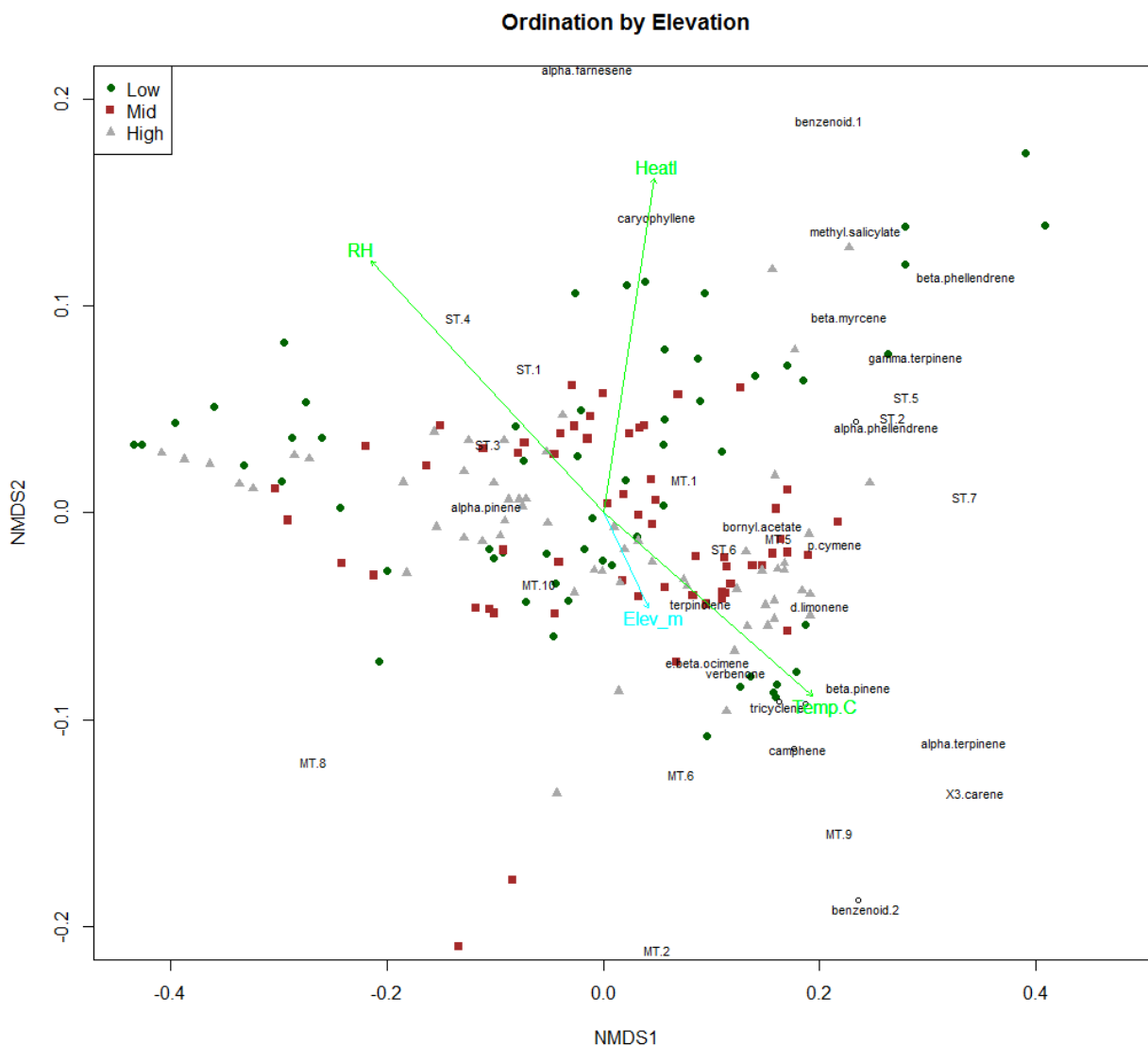
The NMDS ordination of the within-needle compounds (Figure 16) also had a high linear fit ( $R^2 = 0.90$ ) and a medium stress value (0.162), indicating a good representation of the data (Clarke 1993). Sesquiterpenoids clustered in the lower values of both NMDS1 and NMDS2 and along the heat load index vector (Figure 16). The NMDS ordination plot shows a pattern with within-needle chemistry of trees from the highest elevation class distributed along the diagonal of the two NMDS axes (gray triangles in Figure 16), with the mid elevations points clustered centrally, and the lowest elevation trees exhibit more dispersion/variability (Figure 16). NMDS2 was positively

correlated with heat load index and elevation was not significant in the within-needle ordination.

The compound ratio at all sites of  $\alpha$ -pinene/limonene declined significantly as temperature increased ( $P < 0.001$ ,  $R^2 = 0.414$ ) (Figure 17). And the compound ratio of  $\alpha$ -pinene/3-carene declined significantly with temperature increases ( $P < 0.001$ ,  $R^2 = 0.135$ ) (Figure 17). The compound ratio of  $\alpha$ -pinene/ $\beta$ -myrcene declined significantly as temperature increased ( $P < 0.001$ ,  $R^2 = 0.163$ ) (Figure 17)

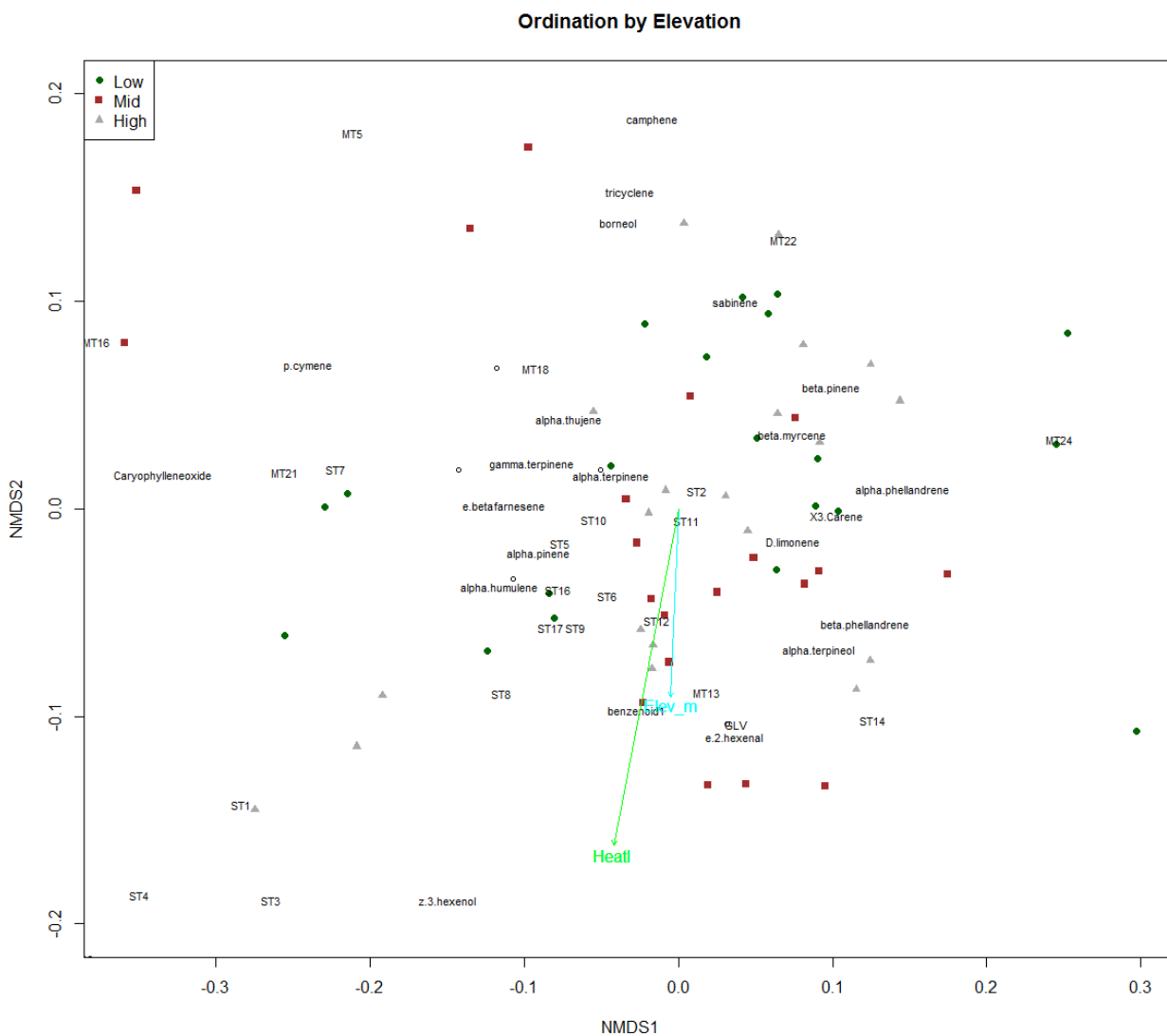
#### *Random Forest Classification of Elevation using VOCs*

Random Forest classification correctly assigned high, mid and low elevation sites in 82.7% of samples (Table 9). When holding back 10% of the sample to validate model classification, the accuracy averaged 83.3% (10 trials), slightly better than the initial out-of-box (OOB) accuracy. Multidimensional scaling (MDS) of the Random Forest model using the 42 VOCs showed strong clustering by elevations class (Figure S1). The most important compounds for differentiating elevation classes were (*E*)- $\beta$ -ocimene,  $\alpha$ -farnesene, B2, MT 8, geranyl acetate, tricyclene, ST 7, linalool, bornyl acetate,  $\gamma$ -terpinene, and caryophyllene (Table 10) which was partly supported by the Kruskal-Wallis-tests (Table 7).

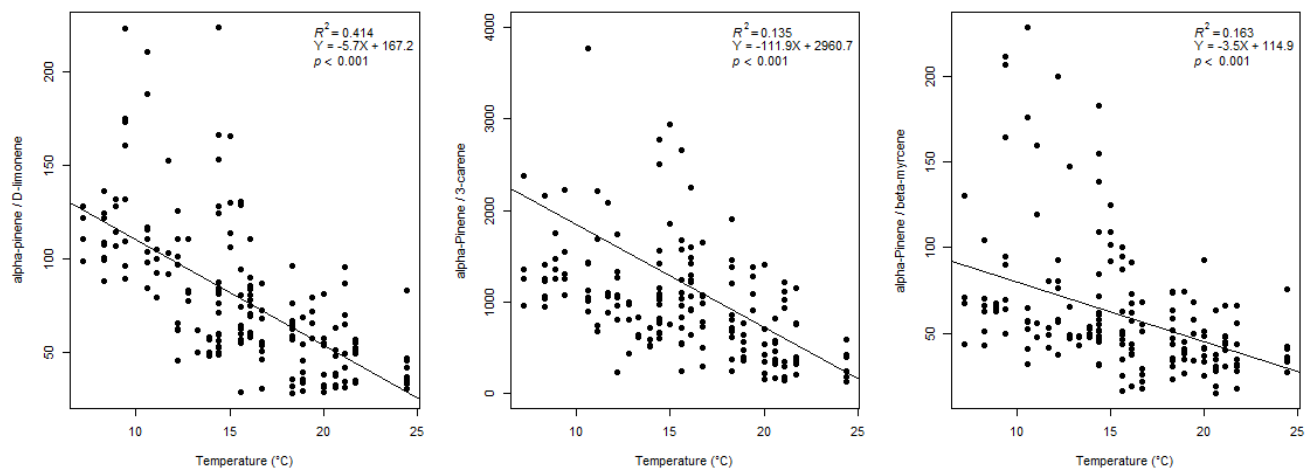


**Figure 15.** Non-linear Multidimensional Scaling (NMDS) ordination plot of the first and second dimensions for the volatile organic compounds (VOCs) emitted (ng per hour per gram;  $n = 132$ ) by Great Basin bristlecone pine. Green vectors are environmental gradients that are significantly correlated ( $\alpha = 0.05$ ) to the VOC ordination. The elevation (m) vector is plotted in light blue and is not significant. Points are tree samples at each time period; colors and shape indicate elevation class. Centroids compounds are overlain in red.

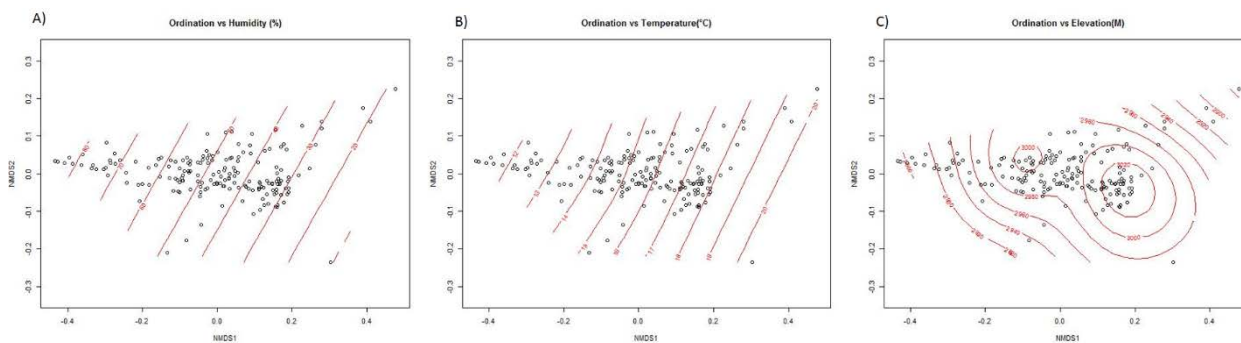




**Figure 16.** Non-linear Multidimensional Scaling (NMDS) plot of the first and second dimensions for within-needle terpenoid concentrations (ng per g;  $n = 60$ ) of Great Basin bristlecone pines growing at low, middle and high elevations. Green vectors are environmental gradients that are significantly correlated ( $\alpha = 0.05$ ) to the terpenoid ordination. The elevation vector (which is not significant) is plotted in light blue. Points are tree samples from each date; colors and shape indicate elevation class.



**Figure 17.** Ratios of A)  $\alpha$ -pinene/limonene, B)  $\alpha$ -pinene/3-carene, and, C)  $\alpha$ -pinene/ $\beta$ -myrcene versus temperature (°C) along Great Basin bristlecone pine transects. Regression coefficients for both ratios are significant ( $p < 0.001$ ).



**Figure 18.** NMDS ordination plots overlaid with a smoothed contours of the environmental variables: A) humidity, B) temperature, and C) elevation.

### *Random Forest Classification of Elevation using VOCs*

Random Forest classification correctly assigned high, mid and low elevation sites in 82.7% of samples (Table 9). When holding back 10% of the sample to validate model classification, the accuracy averaged 83.3% (10 trials), slightly better than the initial out-of-box (OOB) accuracy. Multidimensional scaling (MDS) of the Random Forest model using the 42 VOCs showed strong clustering by elevations class (Figure S1). The most important compounds for differentiating elevation classes were (*E*)- $\beta$ -ocimene,  $\alpha$ -farnesene, B2, MT 8, geranyl acetate, tricyclene, ST 7, linalool, bornyl acetate,  $\gamma$ -terpinene, and caryophyllene (Table 10) which was partly supported by the Kruskal-Wallis-tests (Table 7).

Random Forest classification OOB accuracy for within-needle terpene concentrations was not nearly as good (68% correctly classified), with 30% of the high elevation sites misclassified, 25% of the middle, and 40% of the low sites misclassified. Linalyl acetate, ST21, B1, MT21, sabinene, *cis*-verbenol, and  $\alpha$ -terpineol were the most important variables in classifying elevation using within-needle chemistry.

## **Discussion**

Results show that tree foliage chemical compound measurements change with elevation. More research is needed to understand effects of abiotic stress, and tree susceptibility to insect attack defense and flammability. Yet the factors that control VOC emissions are complex and have long been a research challenge (Peñuelas and Llusà 2003). VOCs also have multiple functions for biotic and abiotic stresses, and emission

**Table 9.** Random Forest model confusion matrix (n=60 for each class)

Observed class	Predicted class			Class error
	High	Middle	Low	
High	48	4	8	0.200
Middle	7	47	5	0.203
Low	4	3	53	0.117

**Table 10.** Importance ranking of volatile compounds in classifying GBBP based on elevation (high, mid, low) based on mean decreasing accuracy (MDA) using Random Forest.

Importance Rank	Compound	Mean Decrease Accuracy
1	( <i>E</i> )- $\beta$ -ocimene	19.15
2	$\alpha$ -farnesene	17.33
3	MT 8	17.28
4	B2	16.22
5	ST 7	16.19
6	linalool	15.33
7	tricyclene	15.20
8	caryophyllene oxide	15.17
9	geranyl acetate	14.44
10	bornyl acetate	13.61
11	$\gamma$ -terpinene	13.47
12	$\beta$ -phellandrene	11.79
13	camphene	11.77
14	$\alpha$ -terpinene	11.47
15	MT 7	11.30
16	$\alpha$ -phellandrene	11.15
17	B1	10.95
18	camphor	10.76
19	<i>p</i> -cymene	10.42
20	$\beta$ -pinene	10.38
21	3-carene	9.98

MT = unidentified monoterpene, B = unidentified benzenoid, ST = unidentified sesquiterpene.

rates vary greatly in response to basic abiotic factors such as light or temperature. In this section, we discuss our major findings and the importance of those findings for science and management of bristlecone pines across environmental gradients.

**How does GBBP chemistry change with elevation, and is this correlated with air temperature, RH, and heat load index, and thus to climate change?**

Previous studies have shown that abiotic stress increases VOC emissions (Tingey et al. 1980, Loreto et al. 1996, Sharkey and Yeh 2001, Duhl 2008, Holopainen and Gershenzon 2010), results from our study correlating VOCs to elevation are more nuanced. Our findings showed that air temperature, heat load index, and relative humidity correlated well with emitted VOCs in the NMDS analysis, however, variability of individual VOCs was high and emissions did not always correspond to elevation (Table 7). For example,  $\beta$ -pinene emissions were highest at the mid elevation site for Spring Mtn, but lowest at the mid elevation site for Cave Mtn. About a quarter of the time, trees growing at the lowest elevations emitted the most total VOCs or trees at the highest elevations emitted the least VOCs suggesting that temperature stress increases VOC emissions (Table 7). The amounts and blends of VOCs emitted by GBBP are correlated with some environmental gradients, while others were not measured (e.g. CO<sub>2</sub>, precipitation, UV, ozone), suggesting that VOC relationships to environmental gradients are complex. The within-needle terpenoid NMDS is correlated with heat load index but not with elevation, suggesting that microsite (e.g. aspect, openness, etc.) variables play an important role in a trees chemical response and factors other than elevation are affecting within needle chemistry.

To further examine elevational effects on VOC composition, we overlaid NMDS with contours of environmental variables (Figure 18). The NMDS axis dissimilarities are more pronounced along the NMDS1 axis and the environmental variables may have driven the VOC amounts. The dissimilarity of compounds along the NMDS1 is perpendicular to the contours of the environmental variables humidity (Figure 16A) and temperature (Figure 18B). If this were not the case, the ordination pattern would either be in a compact cloud, or parallel to the contours. While elevation was not significant and did not drive the ordination like humidity and temperature, there is a clear similarity of points along the elevation contours (Figure 18C). The elevation gradients used in this study were relatively small, only 225 and 520 m difference between low and high sites at Cave Mtn and Spring Mtn, respectively. The typical range of GBBP throughout Nevada is 900 m (2400m-3300m) (Lanner 1983), yet at Cave Mtn, we detected a distinct signal in GBBP over only 225 m. This suggests VOCs are highly sensitive to relatively small elevation changes and to the concomitant environmental changes.

### **Do VOCs emitted from GBBP increase at lower elevations as a proxy for climate warming?**

While there is uncertainty in how climate change will affect mountain regions of the Great Basin, extensive research has modeled probable effects. GBBP have often been used to analyze climate change effects on conifers and reconstruct climate histories. For example, recent unprecedented tree-ring growth in bristlecone pine at the highest elevations and possible causes were presented by Salzer et al. (2009). GBBP chronologies from near treeline show increase in precipitation sensitive tree rings (similar

to lower elevation GBBP chronologies), yet tree rings do not always correlate well with temperature (Salzer et al. 2014). Dynamical downscaling of Global Climate Models (GCMs) simulate future climate change effects in the Great Basin, and suggest a 2-4° C increase in March-April mean temperatures, 60-100% decrease in mean April snow water equivalent (SWE), yet possibly a 10-20% increase in October-April precipitation by the end of the 21<sup>st</sup> century (Scalzitti et al. 2016). Assuming that GBBP treeline communities are temperature limited, it is reasonable to expect higher stands to more closely approximate the climate conditions that are currently at the mid and low elevations (predicted 2-4° C warmer on average). The GBBP living at lower treeline might experience higher mortality rates due to drought stress, competition pressure, and increased likelihood of fire (Gray and Jenkins 2017).

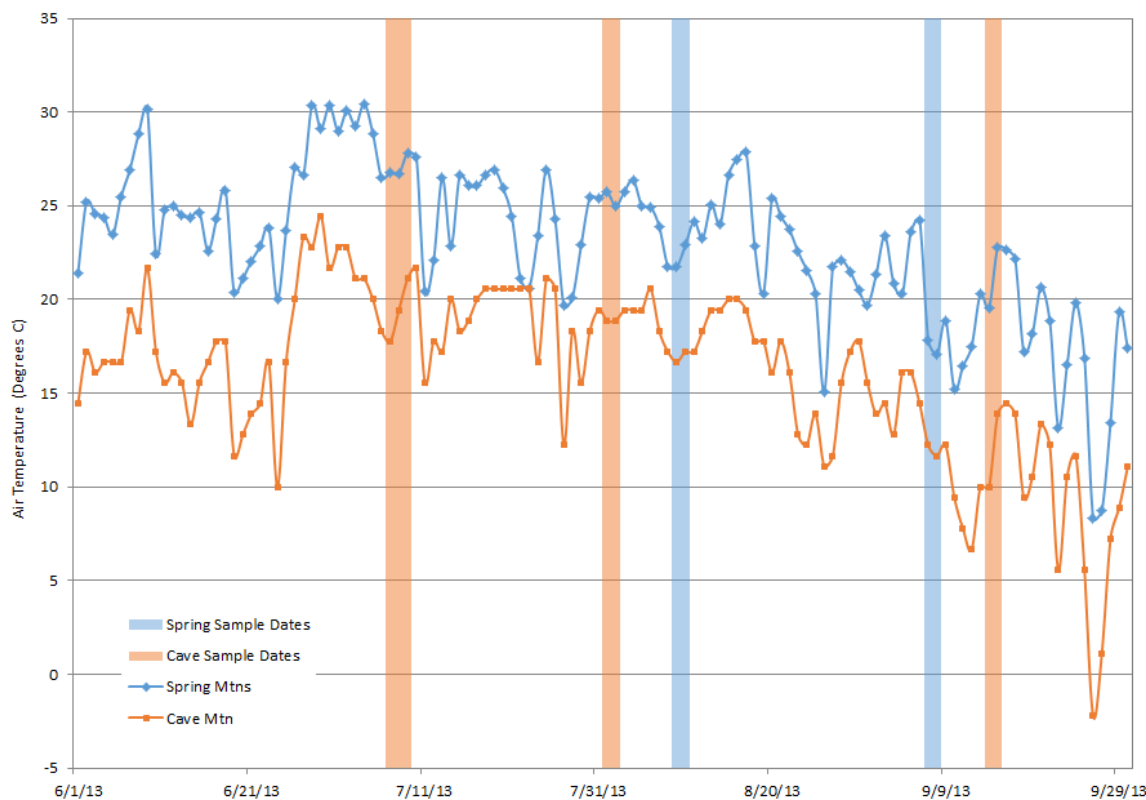
We developed and present an additional method to analyze future climate change effects on GBBP, rather than relying only on tree rings/dendrochronology to understand historical climate changes. NMDS ordination showed that the environmental gradients of temperature, RH, and heat load index all are statistically correlated with the emissions of the 42 identified VOCs (Figure 15 and Figure 16). Our Random Forest classification model was highly accurate at predicting elevation classes from VOCs, demonstrating a distinct chemical signature of trees growing at different elevations. One abiotic factor that changed predictably with elevation was temperature. Temperatures generally were predictable by time of day and elevation. Temperatures were coolest at 10:00, and warmed throughout the day during our sampling. This was not the case in only five of the 45 sample times, when temperatures cooled throughout the day. Temperature patterns along elevation gradients were even more predictable, with only three cases (of 45) in

which a higher elevation site was warmer than a lower elevation site at the same corresponding sample period (and never by more than 1 °C.) The lowest sites were always considerably warmer, with an average 3.7 °C temperature differences between low to mid elevation sites, and an average 1.4 °C difference between mid to high elevation sites at the same time of day. The consistently warmer lower elevation temperatures may lead to more thermal stresses and could explain the exceptionally high Random Forest prediction accuracy (only 12% misclassified) at the low elevation sites (Table 9). These exceptional accuracies are promising for accepting VOC emissions at different elevations as a proxy for climate warming.

Because of the high requirement of photosynthetic carbon for terpene synthesis (Loreto et al. 1996), terpenes can be relatively expensive for a tree to synthesize and store (Lewinsohn et al. 1991, Niinemets 2004, Peñuelas and Staudt 2010), and increased terpene production at lower elevations, which approximates climate change induced heat stress, could reduce tree productivity. Examples of abiotic stresses include extreme temperatures, drought, and wildfires, all of which are factors to GBBP. Abiotic stress is an integral part of climate change which has a wide range of uncertain impacts on plants and trees. A stress event (e.g. extremely hot days) could deplete monoterpenes stored within needles and exhaust reserves (Kravitz et al. 2016). Kravitz et al. (2016) suggest that temperature stress before VOC sampling may affect which terpenes are available, which in turn may provide a potential “early warning” of stress to the ecosystem. There was a period of high temperatures preceding the July sample period and lower temperatures preceding September (Figure 19). These preceding weather events could affect the VOC emissions and available within needle terpenes, and might explain the



monthly differences of VOC emissions observed in the sample (Figure S2). The temperatures at our two sites (approximately the northern and southern extents of GBBP distribution) are highly correlated with each other (Figure 19). The southern Spring Mtn site was about 5 °C warmer than the Cave Mtn site. There is a need to assess tree chemical responses to these environmental changes, and the connection between organism-level stress responses and broader atmosphere-scale studies. These responses are an instrumental gap between atmospheric chemistry and ecosystem studies (Kravitz et al. 2016).



**Figure 19.** Maximum daily temperatures at Cave Mtn SNOTEL station (39.17°, -114.62°; 3224m) and Lee Canyon weather station, Spring Mtns (36.33°, -115.66°; 2947m) study sites. Dates that VOCs were sampled are denoted as shaded bars. Temperatures between sites are highly correlated ( $R^2 = 0.78$ ), with the Spring Mtns consistently warmer by ~5°C.

### **Can we apply understanding of GBBP response to elevational gradients to interpret potential susceptibility to threats like fire or mountain pine beetles?**

Ratios of  $\alpha$ -pinene to other VOCs are important to individual tree defense (Huber et al. 2000, Pureswaran et al. 2004), and previous studies have shown that limonene affected mountain pine beetle attraction (Gray et al. 2015). Monoterpenes are essential co-attractants for mountain pine beetle aggregation pheromones. Yet pine monoterpenes are also toxic physiologically to bark beetles at high vapor concentrations and are an important component of pine defense to insects and fungus (Seybold et al. 2006). In this paper, we demonstrate a significant decrease in ratios of  $\alpha$ -pinene/limonene,  $\alpha$ -pinene/3-carene, and  $\alpha$ -pinene/ $\beta$ -myrcene with increasing temperature (Figure 17). We found 13 compounds (and 3 unidentified monoterpenes) that significantly increase with temperature ( $\alpha = 0.05$ , 11 compounds  $\alpha < 0.001$ ) (Table S1). Of these, several have been shown to interact with mountain pine beetle.  $\beta$ -pinene,  $\beta$ -myrcene, 3-carene, *p*-cymene,  $\beta$ -phellandrene, limonene, (*E*)- $\beta$ -ocimene, and  $\alpha$ -terpinene all induce an antenna response in mountain pine beetle (Huber et al. 2000, Pureswaran et al. 2004).  $\beta$ -myrcene, 3-carene, and  $\beta$ -phellandrene increase flight response to aggregation pheromones (Miller and Borden 2000). And many studies have shown  $\beta$ -myrcene to be an attractant to mountain pine beetle (Pitman 1971, Billings et al. 1976, Borden et al. 1983, Conn et al. 1983, Miller and Lindgren 2000, Pureswaran and Borden 2005). The decrease in ratios of  $\alpha$ -pinene/limonene and  $\alpha$ -pinene/3-carene could make GBBP more closely resemble limber pine as temperatures increase, and mountain pine beetle (which is not strongly attracted to GBBP) could start investigating GBBP as a potential host. The compound ratios from limber pine in Gray et al. (2015) are  $\alpha$ -pinene/3-carene =  $292 \pm 82$ , and  $\alpha$ -

pinene/limonene =  $21 \pm 5$ , these values are found only at the warmest temperatures for GBBP (Figure 17).

Changes in tree chemistry can have important implications for wildfire and foliage flammability. Page et al. (2012) found the individual terpene compounds within foliage that affect flammability parameters (time to ignition, temperature at ignition, and maximum rate of mass loss) are  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene, (*E*)- $\beta$ -ocimene, *p*-cymene, camphene, and tricyclene (along with unknown terpenes). In our regression analysis, the within needle volatile terpene emission rates of  $\alpha$ -pinene,  $\beta$ -pinene and  $\beta$ -myrcene were significant with elevation or heat load index (Table S2), suggesting that at lower and warmer sites these compounds contribute to increased potential flammability. The increase of these three flammable terpenes at the lowest elevations and sites with the highest heat load index suggests that along the margins of the lower tree lines, time to ignition, temperature at ignition, and maximum rate of mass loss could create forests that are more prone to mortality in the event of fire.

## Conclusions

Plants have control over the compounds and amount of VOCs emitted (Widhalm et al. 2015). The production of monoterpenes might offer relief from temperature stress to plants (Peñuelas and Llusà 2003). Thermotolerance has been observed in monoterpene emissions from a Mediterranean oak species (*Quercus ilex*) (Loreto et al. 1998). Monoterpenes and isoprenes protect plant tissues and membranes from oxidative damage produced under high temperatures (Zeidler et al. 1997). It is likely that oxidative and thermal stresses are relieved in the presence of volatile terpenes (Loreto and Schnitzler 2010). When we examined two of the most important variables for prediction in the

Random Forest model (*(E)*- $\beta$ -ocimene and  $\alpha$ -farnesene) (Table 10), the emissions of these compounds at the highest elevation sites was 30% to 50% that of the low sites, this suggest that low elevation trees might be experiencing oxidative stress. Finally, methyl salicylate is thought to provide defense from oxidative and thermal stresses (Loreto and Schnitzler 2010). Again, we observed much lower emissions of methyl salicylate at the highest elevations from both sites (Table 7), suggesting lower elevations trees are experiencing stress. This could offer GBBP protection from higher heat stress at the lower sites. Because individual trees have control over which compounds and how much VOCs are emitted, oxidative and thermal stress could explain why VOCs were more predictive than within-needle terpenes due to VOCs being more sensitive and changing more rapidly in response to the environment than within-needle chemistry.

Terpenes might also play a role in tree longevity in addition to adaptation to abiotic stress and benefits as defenses. For example, terpenes and resins might provide resistance to wood decay (LaMarche 1969, Mourant et al. 2007, Brutovská et al. 2013) leader to great longevity. Also, energy partitioning between defensive investments and growth in woody plants contribute to longevity, suggesting that increasing a tree's life-span should require increased energy invested in protective measures such as thick bark and defensive chemicals (Loehle 1988). Increased investment in such defenses, however, would slow down growth rate, thereby raising the mortality rate for juveniles in competition for height growth. This is the case with GBBP, which primarily grow on dry, nutrient-poor soils, and conditions favorable to seed germination and growth are infrequent (Lanner 1983).

In conclusion, we found that GBBP VOCs differed with elevation, and were correlated with air temperature, RH, and heat load index, while within-needle did not correlate as well. These changes along environmental gradients may be a response to biotic or abiotic stress. This research improves understanding of VOC emissions as a physiological tree response to environmental gradients and helps inform land managers about forests threatened by native and non-native pests and pathogens, increased threat of fire, and changing distribution patterns from climate change. We also improve methods to reliably assess and predict tree resiliency with climate change.

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## Supplemental

**Table S1.** Parameter estimates and goodness of fit statistics for linear regression models of compounds vs temperature, and linear regression models of ratios of  $\alpha$ -pinene/compound.

Compound	Compound ~ Temperature					( $\alpha$ -pinene/compound) ~ Temperature				
	Coefficient	Intercept	P value		R <sup>2</sup>	Coefficient	Intercept	P value		R <sup>2</sup>
limonene	0.23	0.72	0.000	***	0.09	-5.65	166.57	0.000	***	0.42
MT 4	0.07	-0.41	0.000	***	0.12	-111.62	2649.15	0.000	***	0.31
terpinolene	0.09	0.62	0.018	*	0.03	-11.89	381.81	0.000	***	0.3
MT 3	0.02	-0.06	0.000	***	0.07	-256.91	6281.57	0.000	***	0.29
$\gamma$ -terpinene	0.05	-0.38	0.000	***	0.09	-198.67	4897.61	0.000	***	0.28
MT 5	0.02	-0.08	0.000	***	0.09	-360.01	7985.99	0.000	***	0.27
bornyl acetate	0.14	-1.1	0.001	**	0.06	-58.83	1546.22	0.000	***	0.24
$\alpha$ -terpinene	0.02	-0.12	0.000	***	0.07	-607.82	14568.31	0.000	***	0.23
MT 9	0	-0.03	0.000	***	0.13	-3959.83	88980.05	0.000	***	0.20
ST 2	0.01	-0.06	0.000	***	0.19	0.01	-0.06	0.000	***	0.19
$\beta$ -myrcene	0.29	1.27	0.001	**	0.06	-3.51	115.34	0.000	***	0.17
3-carene	0.03	-0.15	0.000	***	0.16	-111.89	2960.85	0.000	***	0.14
B1	0.14	-1.24	0.000	***	0.12	0.14	-1.24	0.000	***	0.12
MT 10	0.02	-0.1	0.048	*	0.02	-832.38	19956.78	0.000	***	0.1
<i>p</i> -cymene	0.06	0.56	0.040	*	0.02	-24.34	695.91	0.000	***	0.1
methyl salicylate	0.03	-0.1	0.000	***	0.09	0.03	-0.1	0.000	***	0.09
$\beta$ -pinene	2.29	5.92	0.018	*	0.03	-0.37	14.4	0.000	***	0.09
ST 4	0.01	-0.02	0.006	**	0.04	-1742.24	42189.33	0.000	***	0.08
ST 6	0	-0.02	0.000	***	0.07	0	-0.02	0.000	***	0.07
$\beta$ -phellandrene	1.72	12.34	0.003	**	0.05	-0.75	22.58	0.001	**	0.06
MT 7	0.01	-0.07	0.001	**	0.06	-4175.78	83363.2	0.001	**	0.06
ST 5	0	0	0.002	**	0.05	0	0	0.002	**	0.05
( <i>E</i> )- $\beta$ -ocimene	0.01	0.08	0.037	*	0.02	-142.25	4303.09	0.004	**	0.05
verbenone	0.01	0.01	0.006	**	0.04	0.01	0.01	0.006	**	0.04
ST 7	0.02	-0.18	0.022	*	0.03	0.02	-0.18	0.022	*	0.03
MT 6	0.01	-0.03	0.022	*	0.03	0.01	-0.03	0.022	*	0.03

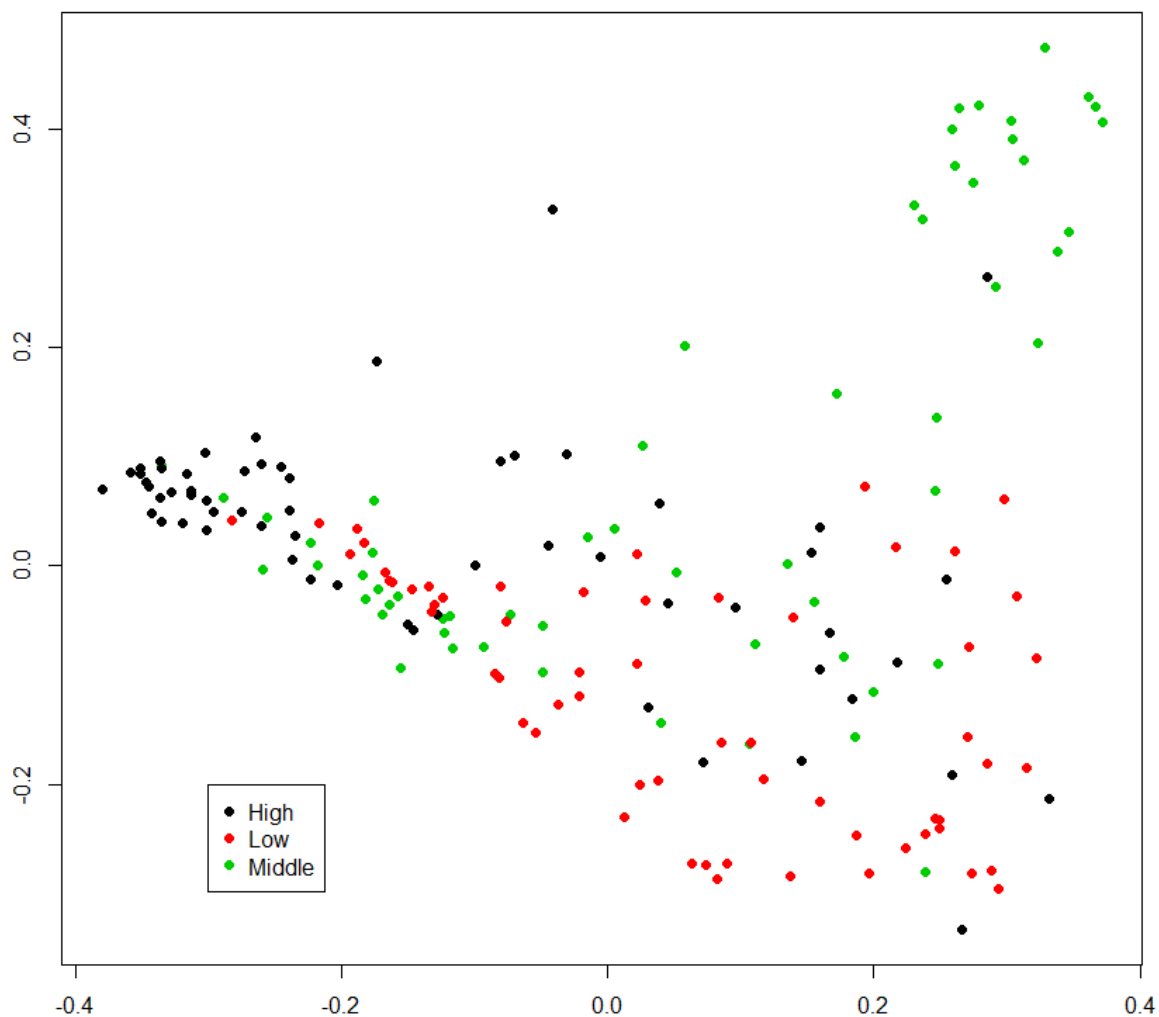
**Significance codes:**  $p < 0.001 = ***$ ,  $p < 0.01 = **$ ,  $0.05 = *$ .

MT = unidentified monoterpene, B = unidentified benzenoid, ST = unidentified sesquiterpene.

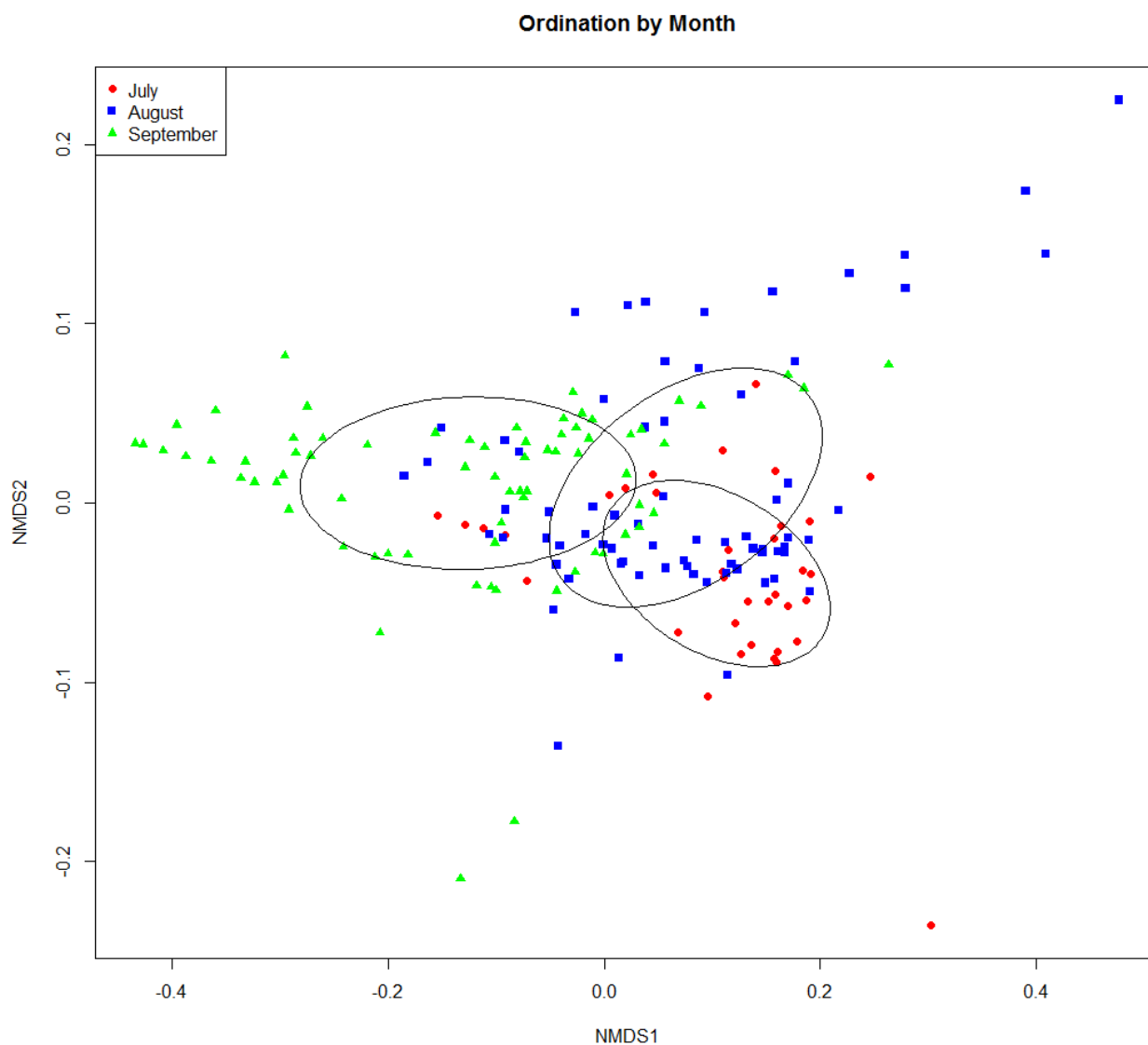
**Table S2. Regression of flammable within needle compounds** -- Parameter estimates and goodness of fit statistics for linear regression models of within needle terpenoid concentrations ( $\mu\text{g/g}$ ) vs temperature and within needle compounds vs Heat load index

Compound	Elevation ~ compound					Heat load index ~ compound				
	Coefficient t	Intercept t	P value		R <sup>2</sup>	Coefficient t	Intercept	P value		R <sup>2</sup>
$\alpha$ -pinene	1.67	-2992	0.006	**	0.12	2214	145.5	0.0003	***	0.21
$\beta$ -pinene	0.47	-71.5	0.04	*	0.07	NS	NS	NS		
$\beta$ -myrcene	NS	NS	NS			214	-4.6	0.003	**	0.14
(E)- $\beta$ -ocimene	NS	NS	NS			NS	NS	NS		
p-cymene	NS	NS	NS			NS	NS	NS		
camphene	NS	NS	NS			NS	NS	NS		
tricyclene	NS	NS	NS			NS	NS	NS		

**Significance codes:**  $p < 0.001 = ***$ ,  $p < 0.01 = **$ ,  $0.05 = *$ .



**Figure S5.** The metric multi-dimensional scaling representation for the proximity matrix of the Random Forest model classifying environment of GBP volatiles at high, mid and low elevations. The proximity matrix measures among the input (based on the frequency that pairs of data points are in the same terminal nodes), how far apart (relatively) clusters are from one another.



**Figure S6.** Non-linear Multidimensional Scaling (NMDS) plot of the first and second dimensions for within-needle terpene concentrations (ng per g;  $n = 60$ ) of Great Basin bristlecone pines. Points are tree samples from each date; colors and shape indicate month. Black ellipsoids are centered on month.

## CHAPTER 5

MOUNTAIN PINE BEETLES USE VOLATILE CUES TO LOCATE HOST LIMBER  
PINE AND AVOID NON-HOST GREAT BASIN BRISTLECONE PINE<sup>2</sup>**Abstract**

The tree-killing mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is an important disturbance agent of western North American forests and recent outbreaks have affected tens of millions of hectares of trees. Most western North American pines (*Pinus* spp.) are hosts and are successfully attacked by mountain pine beetles whereas a handful of pine species are not suitable hosts and are rarely attacked. How pioneering females locate host trees is not well understood, with prevailing theory involving random landings and/or visual cues. Here we show that female mountain pine beetles orient toward volatile organic compounds (VOCs) from host limber pine (*Pinus flexilis* James) and away from VOCs of non-host Great Basin bristlecone pine (*Pinus longaeva* Bailey) in a Y-tube olfactometer. When presented with VOCs of both trees, females overwhelmingly choose limber pine over Great Basin bristlecone pine. Analysis of VOCs collected from co-occurring limber and Great Basin bristlecone pine trees revealed only a few quantitative differences. Noticeable differences included the monoterpenes 3-carene and D-limonene which were produced in greater amounts by host limber pine. We found no evidence that 3-carene is important for beetles when selecting trees, it was not attractive alone and its addition to Great Basin bristlecone pine VOCs did not alter female

<sup>2</sup>Suggested Citation: Gray CA, Runyon JB, Jenkins MJ, Giunta AD (2015) Mountain Pine Beetles Use Volatile Cues to Locate Host Limber Pine and Avoid Non-Host Great Basin Bristlecone Pine. PLoS ONE 10(9): e0135752. doi:10.1371/journal.pone.0135752

selection. However, addition of D-limonene to Great Basin bristlecone pine VOCs disrupted the ability of beetles to distinguish between tree species. When presented alone, D-limonene did not affect behavior, suggesting that the response is mediated by multiple compounds. A better understanding of host selection by mountain pine beetles could improve strategies for managing this important forest insect. Moreover, elucidating how Great Basin bristlecone pine escapes attack by mountain pine beetles could provide insight into mechanisms underlying the incredible longevity of this tree species.

## **Introduction**

The mountain pine beetle (MPB; Coleoptera: Curculionidae; *Dendroctonus ponderosae* Hopkins) is one of the most ecologically and socioeconomically important forest insects in North America. Outbreaks of this native insect during the early 21st century have been extensive, with over 3.5 million hectares of tree mortality in 2009 alone (Man, 2010). Such outbreaks can have important consequences for wildlife (Saab et al., 2014), forest carbon dynamics (Hansen, 2014), nutrient cycling (Griffin et al., 2011), wildfires (Jenkins et al., 2014), and have contributed to the rapid decline of some high elevation tree species (Gibson et al., 2008; Logan et al., 2010).

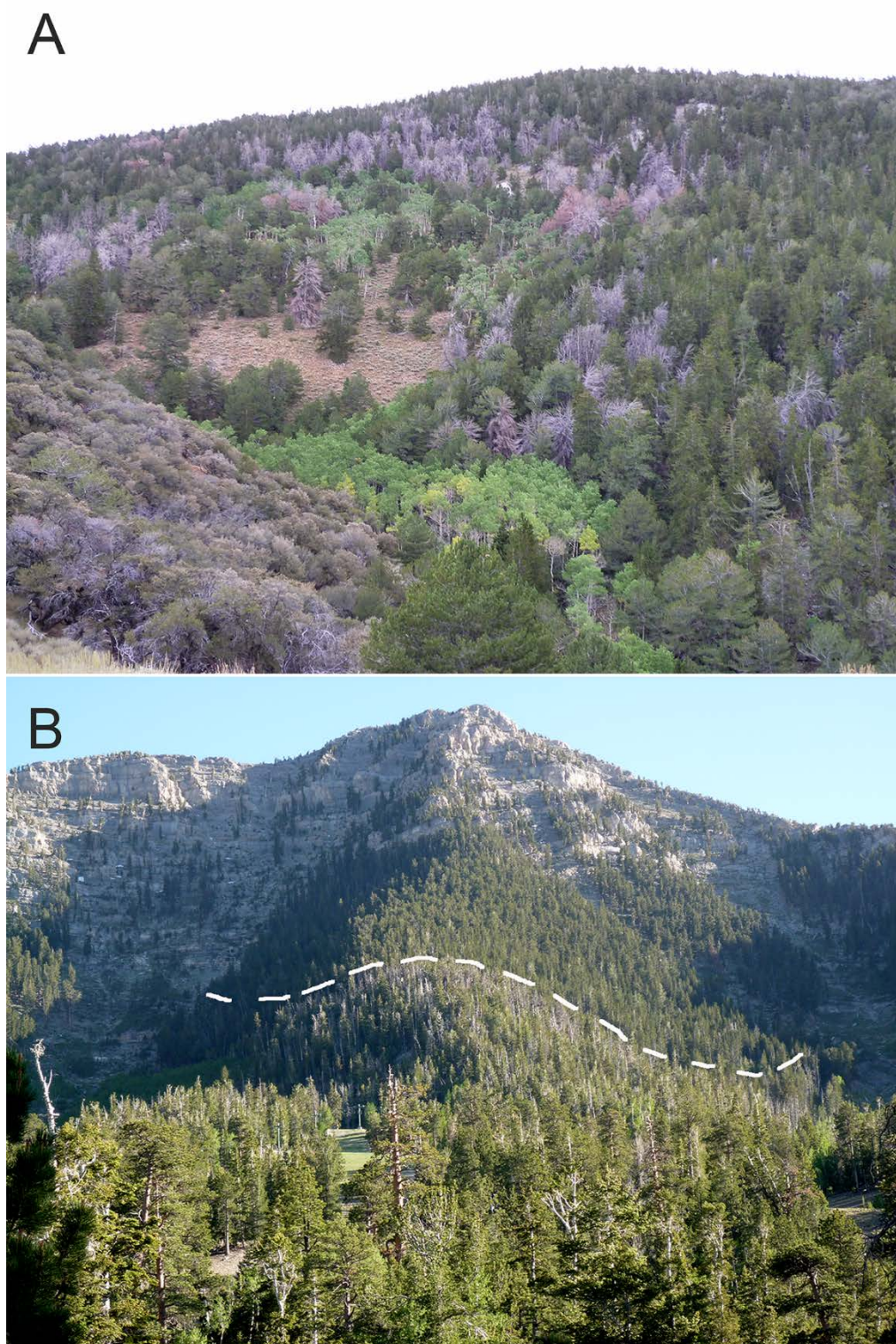
MPBs kill trees by attacking *en masse* using a complex system of volatile semiochemicals involving multiple beetle-produced aggregation and anti-aggregation pheromones and host-produced kairomones (Progar et al., 2014). Once in contact with a suitable host, pioneering females initiate mass attacks by oxidizing the host-produced monoterpene  $\alpha$ -pinene to produce the aggregation pheromone verbenol (Progar et al.,



2014). Males arrive and produce *exo*-brevicomin which attracts more beetles. Host-produced monoterpenes including  $\alpha$ -pinene (Pitman et al., 1968), myrcene, and terpinolene (Borden et al., 2008) synergize the aggregation pheromones. In the latter stages of a mass attack, increased production of the anti-aggregation pheromone verbenone (via oxidation of verbenol) terminates host colonization (Hunt et al., 1989). Despite having a good understanding of the sophisticated chemical ecology underlying mass attacks, less is known about the cues used by pioneering females to locate trees (Safranyik and Carroll, 2007). The prevailing theory is that during the pre-aggregation phase females locate host trees using visual cues or through random landings (Progar et al., 2014; Safranyik and Carroll, 2007). Studies have reported MPB attraction to dark silhouettes and large, tree-shaped cylinders (Billings et al., 1976; Shepherd, 1966) suggesting a role for visual cues. Other studies have indicated that pioneering females intercept hosts at random which explains MPB's preference for large diameter trees due to their larger surface area (Burnell, 1977; Hynum and Berryman, 1980). Conversely, there is evidence for the use of long distance sensing using volatile organic compounds (VOCs) by MPBs (Borden et al., 1986; Raffa et al., 1993; Wood, 1982). Plant VOCs emitted by trees are known to be used in host location by other bark beetle species (Rudinsky, 1966), suggesting they might be similarly used in host location by MPBs.

In this study, we investigated whether pioneering female MPBs use VOCs to choose between the host limber pine (*Pinus flexilis* James) and the non-host Great Basin bristlecone pine (*Pinus longaeva* Bailey). Limber pine is a favored and highly-productive host of MPBs (Langor, 1989) and limber pine forests have experienced dramatic MPB mortality since the 1990s (Man, 2010). In contrast, Great Basin

bristlecone pine has not been shown to be an acceptable host for MPB (Gibson et al., 2008) and concrete records of successful MPB attack are lacking. These two species often occur together as the only tree species growing at or near alpine treeline in the Great Basin and Intermountain West of the USA (the “*P. flexilis*/*P. longaeva* Series” (Youngblood and Mauk, 1985)). These high elevation pine forests provide important ecosystem services (Schoettle, 2004), including stabilizing soil, improving snow retention, pioneering regeneration of alpine sites after fire, habitat for wildlife, and facilitating growth of other tree species (Baumeister and Callaway, 2006). This study was spurred by our observations at several sites in Nevada where these species co-occur that many limber pines were killed by MPB whereas neighboring bristlecone pines were unattacked (Fig. 20).



**Fig. 20.** Photographs of limber pine (*Pinus flexilis*) and Great Basin bristlecone pine (*Pinus longaeva*) forests (a) on Cave Mountain in east-central Nevada, and (b) in the

Spring Mountains in southern Nevada. These tree species co-occur in nearly equal abundance on and near the top of Cave Mountain (a), the dead trees (gray trees) are mountain pine beetle-killed limber pine. In the Spring Mountains (b), these tree species co-occur below dashed line, note many dead limber pine trees (gray trees), but a nearly pure stand of un-attacked bristlecone pine occurs above the dashed line.

The objectives of this work were to 1) collect and analyze VOCs of co-occurring limber and bristlecone pines as potential foraging cues for the MPB, and 2) assess the behavioral responses of female MPBs to limber and bristlecone pine VOCs in a Y-tube olfactometer. We also explored the role of candidate individual volatile compounds in the behavioral response of MPBs. We hypothesized that VOCs differ between tree species and serve as a readily available cue that foraging MPBs can use in host finding.

## **Materials and Methods**

### **Source and handling of insects and plants**

Adult mountain pine beetles were obtained from two locations infested with MPB (separated by about 90 km) by felling two mature lodgepole pines (*Pinus contorta* Dougl.) infested with MPB larvae from the Bear River Range of Northern Utah (41.9705°, -111.5406°, elevation 2200 m) and from the Caribou-Targhee National Forest in Southern Idaho (42.7772°, -111.2735°, elevation 2040 m). Sections from the bole of the trees (~60 cm long) were transported to the US Forest Service's Rocky Mountain Research Station laboratory in Logan, UT and ends sealed with paraffin wax to reduce desiccation. The sections were placed in Percival incubator cabinets (12 hours of light per 24-hour cycle) at room temperature (ca. 21° C) to facilitate larval development to the adult stage (approximately 70 to 80 days). Emerging adults were collected daily and placed in petri dishes with moistened filter paper and stored in a refrigerator at

approximately 3° C until use. Gender was determined using characters on the seventh abdominal tergite (Lyon, 1958). Virgin females aged 5-15 days were randomly selected for Y-tube trials.

Foliage of limber pine and Great Basin bristlecone pine used in bioassays was collected from Notch Peak, UT (39.1486°, -113.4060°, elevation 2788 m) and Wheeler Peak, NV (38.9991°, -114.2990°, elevation 3181 m) by cutting branches approximately 50 cm in length from a randomly selected bristlecone pine and limber pine from each site, and refrigerating them in sealed plastic bags at approximately 3° C until use. VOCs from these samples were collected and analyzed at time of use as described below. The USDA Forest Service, Humboldt-Toiyabe and Caribou-Targhee National Forests, and the Utah Division of Forestry, Fire, and State Lands granted permission for use of all field sites.

### **Collection and analysis of VOCs**

VOCs were collected from co-occurring limber pine and bristlecone pine trees of similar size in the Spring Mountains near Las Vegas, Nevada (June 2013; 36.2935°, -115.6861°, elevation 2910 m) and on Cave Mountain near Ely, Nevada (August 2013; 39.1623°, -114.6109, elevation 3220 m). Trees of similar size were selected for sampling and VOCs were collected from lower branches ( $\leq 3$  m above ground) which correspond to the heights at which dispersing MPBs fly [9]. The mean height of limber pine trees sampled was  $12.1 \pm 0.7$  m and mean diameter at breast height (dbh) was  $102 \pm 11.6$  cm. The mean height of bristlecone pine trees sampled was  $12.9 \pm 0.61$  m and mean dbh was  $85 \pm 7.9$  cm). Field collection of VOC emissions followed procedures described in Page et al. (Page et al., 2014, 2012). Approximately 70 cm of the apical portion of each branch

on each tree was enclosed in a clear Teflon bag (50 cm wide x 75 cm deep; American Durafilm Co., Holliston, MA) and air was pulled (0.5 L/min) through VOC traps that contained 30 mg of the absorbent material HayeSep-Q (Restek, Bellefonte, Pennsylvania) using an automated portable VOC collection system (Volatile Assay Systems, Rensselaer, NY). VOCs were collected for 30 minutes after which the foliage from the enclosed portion of the branch was weighed to obtain a fresh weight.

VOCs were eluted from traps using 200  $\mu$ l of dichloromethane and 1,000 ng of n-nonyl-acetate added as an internal standard. Samples were analyzed using an Agilent 7890A gas chromatograph (GC) coupled with a 5975C mass spectrometer and separated on a HP-1ms (30 m x 0.25 i.d, 0.25  $\mu$ m film thickness) column; helium was used as the carrier gas. The GC oven was maintained at 35°C for 3 minutes and then increased by 5°C per minute to 125°C, then 25°C per minute to 250°C. Quantifications were made relative to internal standards using ChemStation software (Agilent Technologies, Wilmington, DE), and identifications of compounds confirmed by comparing retention times and mass spectra to commercial standards. Measurements of VOC emissions (ng per hour per gram) were on a fresh weight basis.

Statistical analyses for VOCs were performed using the non-parametric Kruskal-Wallis one-way analysis of variance (Kruskal and Wallis, 1952) using R statistical software (R Development Core Team, 2012) to identify compounds with significant ( $P \leq 0.05$ ) differences, and to test whether samples originate from the same distribution.

### **Y-tube olfactometer trials**

We used a Y-tube olfactometer to investigate the response of adult female MPBs to airborne cues, following the methodology of others (e.g., (Daisy et al., 2002), (Piesik et al., 2008), (Liu et al., 2011)). Y-tube olfactometers have been widely used to examine the role of volatile cues in host location by flying arthropods, including bark beetles (Liu et al., 2011). The Y-tube system (Sigma Scientific LLC, Micanopy, FL, USA) consisted of a 2-port Clean Air Delivery System (CADS-2P), inline odor source chambers (custom made), and a glass Y-tube (YT-2425). The CADS-2P provided flow-controlled, purified air via 0.64 cm outer diameter (OD) Teflon tubing to the odor source chambers (one chamber upwind of each Y-tube arm) and then the Y-tube. The glass odor source chambers were 19 cm long with 5.5 cm inner diameter (ID); the upstream end was sealed with a removable 5.5 cm OD Teflon o-ring endcap with 0.64 cm OD tubing connector, and the downstream end tapered to accept 0.64 cm OD Teflon tubing. The glass Y-tube had a 2.4 cm ID with 24/25 inner ground-glass joints on all ends, a 16 cm lower arm, and 10 cm upper arms that branch at an inner angle of approximately 75°. A specimen adapter (SA-2425), attached via ground-glass joint to the bottom of the Y-tube was used to introduce beetles to the airstream.

Trials were conducted in a greenhouse at temperatures between 20-27° C. Mountain pine beetles are positively phototactic (Shepherd, 1966), so to assure balanced lighting we placed the Y-tube in an open-top box that was lined with black felt (55 tall x 55 wide x 90 cm long). A greenhouse light (400W metal halide, Sylvania Inc., Manchester, NH, USA) was centered 1 m above and just beyond the apex of the Y-tube. To facilitate beetle walking, we placed a 16 cm long, 2 mm diameter metal wire in the

bottom of the Y-tube, extending from the introduction point to the junction of the 'Y'. The odor sources, 20 g of plant material (10 – 15 cm branches with attached needles) and/or rubber septa treated with VOCs, were placed in odor source chambers and an individual insect introduced via specimen adapter at the bottom of the Y-tube. Airflow was set at 0.5 L/min for all trials. Trials ended when the insect moved past the midpoint of the bifurcation in the Y-tube and 5 cm into one of the arms of the 'Y' or after 10 minutes if the insect did not respond ("no responses"). Individual beetles were only used once. The odor source chambers were alternated every five trials. For each odor source, trials were run until at least 25 choices were made (i.e. excluding no responses).

We used rubber septa treated with synthetic VOCs to test how individual compounds affect beetle behavior following methods outlined by Runyon et al. (Runyon et al., 2006). We chose to examine 3-carene and D-limonene because the relative amounts of these compounds differed greatly between bristlecone and limber pine, and they were commercially available in nearly pure form. Red rubber septa (6.6 mm O.D., Sigma Aldrich, St. Louis, MO, USA) were treated with 1  $\mu\text{g}$  of either 3-carene (Product No: 21986,  $\geq 98.5\%$  sum of enantiomers, Sigma Aldrich) or D-limonene (Product No: 62118,  $\geq 99\%$  sum of enantiomers, Sigma Aldrich) in *n*-hexane (Macron Chemicals, Center Valley, PA, USA); 500  $\mu\text{l}$  of 200 ng/ $\mu\text{l}$  hexane solution added to each septum. Control septa were treated with 500  $\mu\text{l}$  of *n*-hexane only. Treated and control septa were left in a fume hood at room temperature and release rates checked each day as described above. Release rates of both compounds responded similarly: amounts released mimicked that of limber pine foliage used in Y-tube trials on day 4 after treatment for D-limonene (approximately 80 ng per hr) and day 5 after treatment for 3-carene



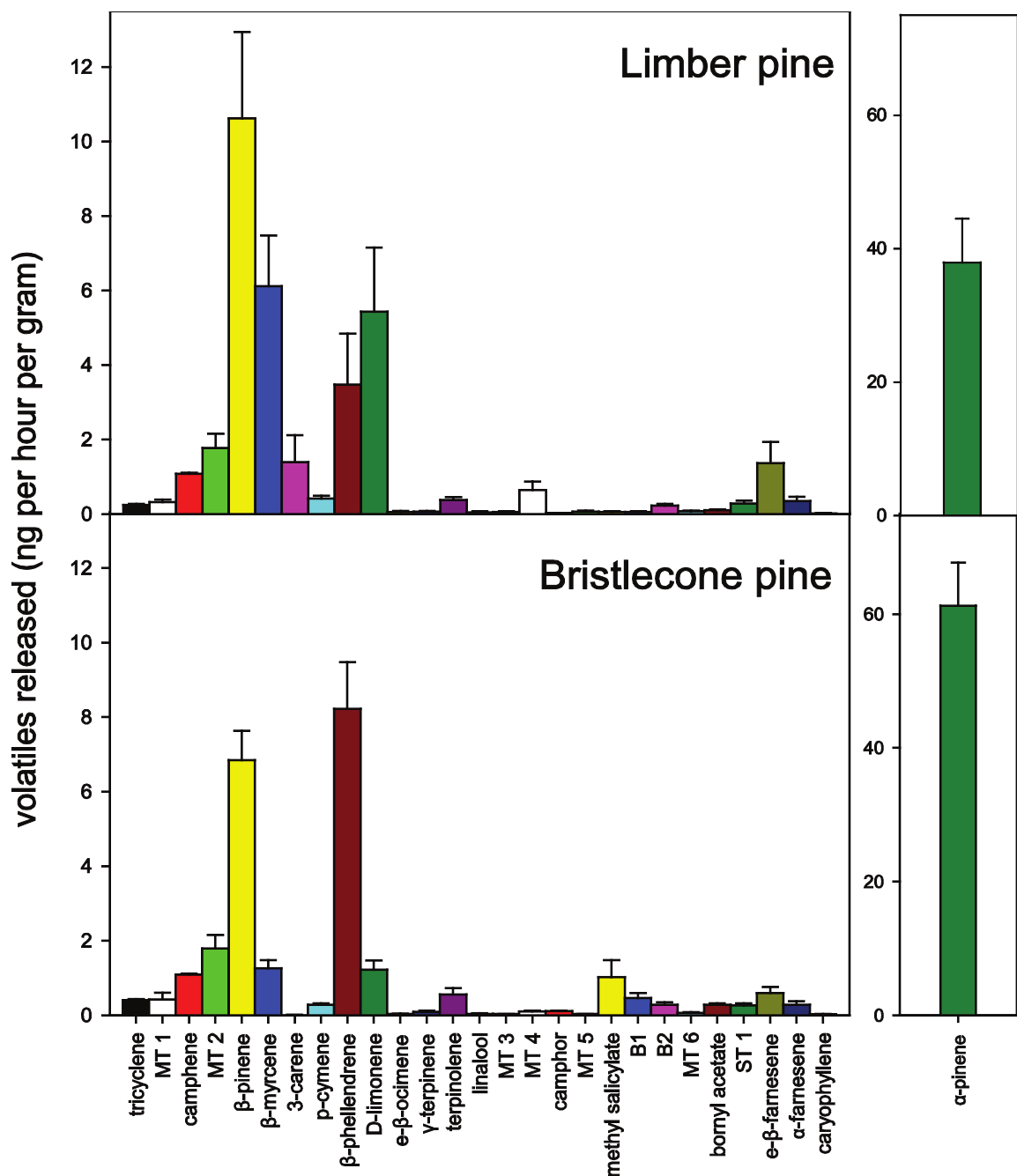
(approximately 50 ng per hr) after treatment. We collected and analyzed a small number of foliage samples with the commercial compound added to verify that the target compound was present and in greater abundance.

Statistical analyses for Y-tube trials were performed using chi-square tests with the Yate's continuity correction for small sample sizes (Sokal and Rohlf, 1981): for each trial we subtracted 0.5 from observed values greater than the expected and added 0.5 to observed values less than the expected.

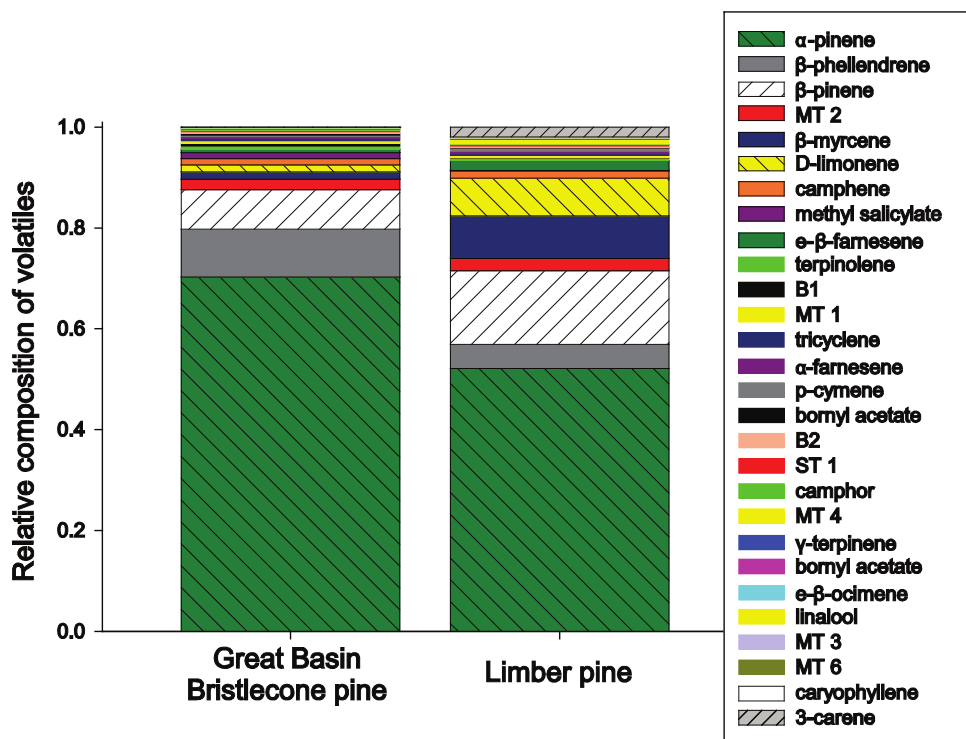
## **Results**

### **Great Basin bristlecone pine and limber pine VOCs**

The VOCs emitted by Great Basin bristlecone pine and limber pine at Cave Mountain were similar. Both species emitted the same 28 VOCs (Fig. 21, Fig. 22 and Table 11) and differed in amounts produced for only ten of these compounds ( $P < 0.05$ ; Table 1). Moreover, the total amount of VOCs released per gram of foliage did not differ between species (Table 11). Monoterpenes dominated the VOC composition of both tree species with  $\alpha$ -pinene being the most abundant followed by  $\beta$ -pinene,  $\beta$ -phellandrene, D-limonene, and  $\beta$ -myrcene (Fig. 21, Fig. 22 and Table 11). A notable difference was the monoterpene 3-carene which was produced by limber ( $1.4 \pm 0.72$  ng per hour per gram) but nearly absent from bristlecone VOCs ( $0.02 \pm 0.003$  ng per hour per gram). The ratios of compounds also varied between species, for example the ratio of  $\beta$ -phellandrene to D-limonene was approximately 1:1 in limber but 7:1 in bristlecone (Fig. 22, Table 11). The amounts and identity of VOCs reported here for Cave Mountain are very similar for co-occurring bristlecone and limber pine trees at a second site in the Spring Mountains near



**Fig. 21.** Total volatiles emitted (ng per hour per gram,  $n = 15$ ) by limber pine (*Pinus flexilis*) and Great Basin bristlecone pine (*Pinus longaeva*) at Cave Mountain, Nevada. These tree species co-occur in nearly equal abundance at this site and many limber pines have been killed by mountain pine beetles whereas bristlecone pines have not been attacked. Note different scale for  $\alpha$ -pinene.



**Fig. 22.** Relative composition of volatiles emitted by co-occurring limber pine (*Pinus flexilis*) and Great Basin bristlecone pine (*Pinus longaeva*) at Cave Mountain, Nevada. Compounds in the legend are listed from most abundant (top) to least abundant (bottom) emitted by Great Basin Bristlecone pine.

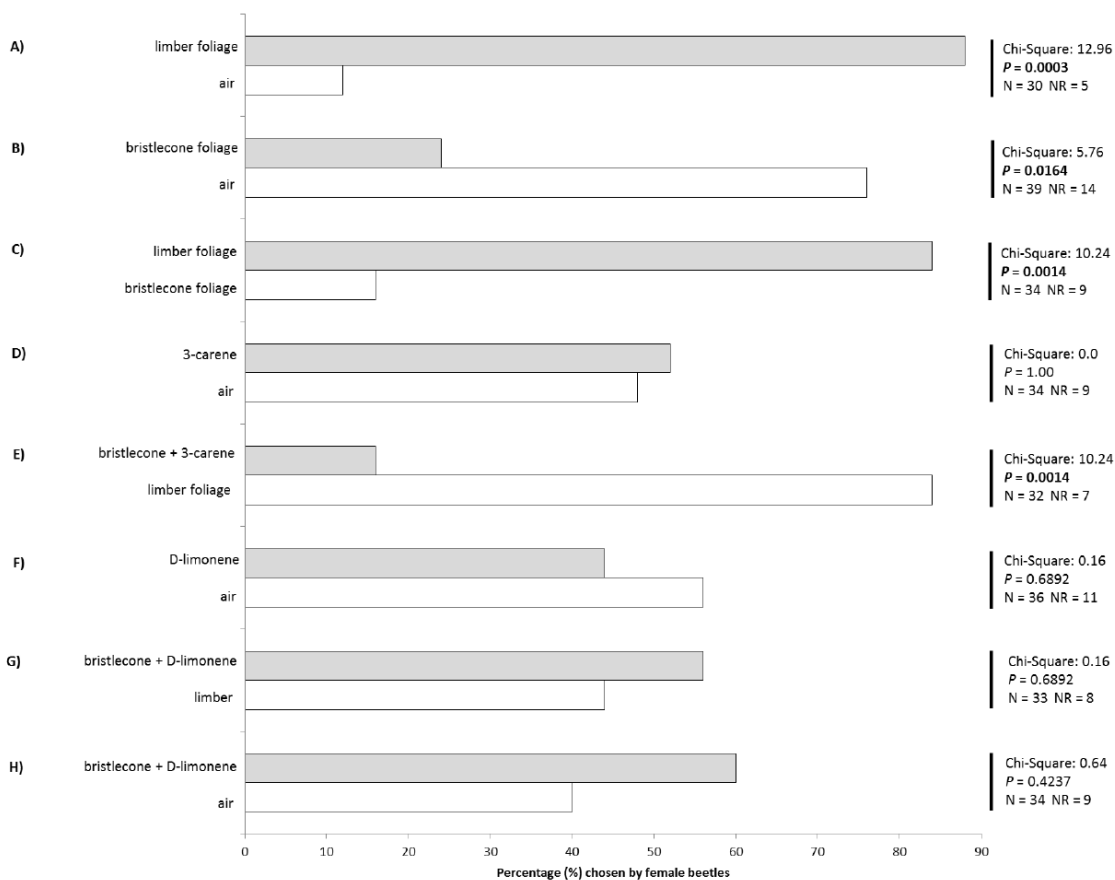
**Table 11.** Volatiles emitted (ng per hour per gram;  $n = 15$ ) by co-occurring Great Basin bristlecone pine (*Pinus longaeva*) and limber pine (*Pinus flexilis*) at Cave Mountain, Nevada. Significant differences are highlighted in bold. MT = unidentified monoterpene, B = unidentified benzenoid, ST = unidentified sesquiterpene.

Compound	Great Basin bristlecone		Limber		P-value
	Mean	SE	Mean	SE	
<b>tricyclene</b>	<b>0.41</b>	<b>0.029</b>	<b>0.25</b>	<b>0.027</b>	<b>0.001</b>
MT 1	0.42	0.181	0.32	0.062	0.290
<b><math>\alpha</math>-pinene</b>	<b>61.25</b>	<b>6.473</b>	<b>37.95</b>	<b>6.533</b>	<b>0.011</b>
camphene	1.09	0.029	1.08	0.028	0.349
MT 2	1.79	0.357	1.77	0.386	0.604
$\beta$ -pinene	6.85	0.784	10.62	2.316	0.481
<b><math>\beta</math>-myrcene</b>	<b>1.26</b>	<b>0.221</b>	<b>6.12</b>	<b>1.357</b>	<b>&lt;0.001</b>
<b>3-carene</b>	<b>0.02</b>	<b>0.003</b>	<b>1.40</b>	<b>0.721</b>	<b>&lt;0.001</b>
p-cymene	0.29	0.034	0.42	0.072	0.254
<b><math>\beta</math>-phellandrene</b>	<b>8.22</b>	<b>1.254</b>	<b>3.47</b>	<b>1.373</b>	<b>0.001</b>
D-limonene	1.22	0.245	5.44	1.716	0.120
e- $\beta$ -ocimene	0.04	0.009	0.05	0.028	0.188
$\gamma$ -terpinene	0.09	0.031	0.07	0.016	0.573
terpinolene	0.56	0.170	0.37	0.083	0.533
linalool	0.03	0.017	0.04	0.037	0.318
MT 3	0.03	0.007	0.06	0.019	0.382
<b>MT 4</b>	<b>0.10</b>	<b>0.025</b>	<b>0.65</b>	<b>0.225</b>	<b>0.001</b>
<b>camphor</b>	<b>0.11</b>	<b>0.014</b>	<b>0.03</b>	<b>0.005</b>	<b>&lt;0.001</b>
MT 5	0.03	0.005	0.07	0.029	0.208
<b>methyl salicylate</b>	<b>1.02</b>	<b>0.459</b>	<b>0.06</b>	<b>0.015</b>	<b>&lt;0.001</b>
<b>B1</b>	<b>0.46</b>	<b>0.137</b>	<b>0.06</b>	<b>0.016</b>	<b>&lt;0.001</b>
B2	0.28	0.067	0.23	0.047	0.633
MT 6	0.07	0.014	0.08	0.018	0.983
<b>bornyl acetate</b>	<b>0.28</b>	<b>0.037</b>	<b>0.11</b>	<b>0.021</b>	<b>&lt;0.001</b>
ST 1	0.26	0.060	0.29	0.080	0.647
e- $\beta$ -farnesene	0.60	0.165	1.37	0.570	0.254
$\alpha$ -farnesene	0.29	0.091	0.35	0.111	0.480
caryophyllene	0.03	0.009	0.02	0.009	0.509
Total volatiles	87.11	7.891	72.76	12.400	0.110

Las Vegas, Nevada (data not shown). We verified that VOCs from foliage used in Y-tube trials were similar to that of intact trees: clipped foliage emitted the same major compounds in approximately the same proportions, only in greater amounts per gram (perhaps due to clipping the branches off trees) (Fig. S7).

### **Behavioral responses of female MPBs to VOCs**

Adult female MPBs overwhelmingly chose the Y-tube arm with limber pine VOCs over the arm with clean air (22 limber vs. 3 air, 5 no responses; Fig. 23A). In contrast, MPB females avoided bristlecone VOCs in favor of clean air (6 bristlecone vs. 19 air, 14 no responses; Fig. 23B). When presented with VOCs from both limber and bristlecone, female MPBs overwhelmingly chose limber VOCs (21 limber vs. 4 bristlecone, 9 no responses; Fig. 23C). We tested a role for 3-carene and D-limonene in the behavioral response by presenting the synthetic VOCs on rubber septa in the Y-tube. 3-carene had no effect on beetle behavior when presented alone (13 3-carene vs. 12 air, 7 no responses; Fig. 23D) or when added to bristlecone pine VOCs (21 limber vs. 4 bristlecone + 3-carene, 7 no responses; Fig. 23E). Similarly, D-limonene alone did not affect MPB behavior (11 D-limonene vs. 14 air, 11 no responses; Fig. 23F). However, addition of D-limonene to bristlecone VOCs negated MPBs strong preference for limber VOCs (11 limber vs. 14 bristlecone + D-limonene, 9 no responses; Fig. 23G) and blocked the ability of MPBs to avoid bristlecone VOCs (15 bristlecone + D-limonene, 10 air, 9 no responses; Fig. 23H).



**Fig. 23.** Behavioral responses of walking female mountain pine beetles (*Dendroctonus ponderosae*) to different odor sources in a Y-tube olfactometer. We used chi-square statistical tests for comparison between the numbers for each choice. NR = no response after 10 minutes. Significant results shown in bold. N = total number of trials (including no responses).

## Discussion

There is some debate about how pioneering female MPBs locate hosts with the dominant hypothesis being a combination of random landings and visual cues followed by direct assessment of host suitability after landing (Progar et al., 2014; Safranyik et al., 2004). The explanation that pioneering females locate trees randomly (Hynum and Berryman, 1980; Vité and Gara, 1962), and/or using sight (Shepherd, 1966) gained support in part because it elegantly explains why large trees are disproportionately

attacked – because they present beetles with the largest landing and visual targets. However, there is evidence in the literature that dispersing female MPBs use VOCs when foraging for hosts. Female MPBs were attracted to cages containing host material but not empty cages in the absence of normal visual cues (Moeck and Simmons, 1991) and antennae of female MPBs are capable of perceiving and responding to host tree VOCs (D. S. Pureswaran et al., 2004a). Moreover, other bark beetle species are known to use VOCs to locate hosts, including other *Dendroctonus* species (Rudinsky, 1966; Sokal and Rohlf, 1981). Here, the results from our study show that female MPBs are strongly attracted to VOCs emitted from limber pine, a preferred host, and are repelled by VOCs emitted from Great Basin bristlecone pine, a non-host. Moreover, female MPBs can distinguish limber and bristlecone pine trees using VOCs and preferentially move toward the former. These findings provide strong evidence that female MPBs use VOCs as cues to locate and select among potential hosts.

The VOCs of limber and bristlecone pine are very similar (Fig. 21), so how do MPBs distinguish between them? We chose two candidate VOCs that differed between species and examined whether they are involved in host choice: the monoterpenes 3-carene and D-limonene. 3-carene alone or addition of 3-carene to bristlecone VOCs to mimic the amount in limber VOCs had no effect on beetle behavior (Fig. 23D-E). However, similar addition of D-limonene to bristlecone VOCs blocked MPB's ability to distinguish between trees species (Fig. 23G). Interestingly, D-limonene alone was not attractive to MPBs (Fig. 23F) suggesting that it is likely the combination or ratio of compounds that provides species-specific information to MPBs. The relative proportion of volatile components in a VOC blend is known to be used in host recognition by some

insect herbivores (Bruce and Pickett, 2011), and experimentally enhancing levels of certain volatile components has been shown to interfere with host location of other herbivore species (Visser and Avé, 1978). In fact, VOCs of many conifer species lack species-specific compounds, suggesting that bark beetles in general might detect differences in the ratios of different compounds to discriminate among tree species (D. S. Pureswaran et al., 2004b). Recent research suggests that *Dendroctonus valens* LeConte, a species related to MPB, use small variations in ratios of VOCs to gauge and select large diameter trees over small diameter trees (Liu et al., 2011). This provides a plausible mechanism by which beetles could measure and choose large host trees using VOCs alone, however, we expect that VOCs and visual cues both contribute to host location and selection by MPBs, as suggested by others (Campbell and Borden, 2009, 2006).

The tree species examined in this study, Great Basin bristlecone pine and limber pine, are climax species that often co-occur as the only trees at or near alpine treeline across much of the Great Basin of North America (Youngblood and Mauk, 1985). Such high elevation ecosystems are of great ecological importance, but are rapidly declining across western North America due to unprecedented outbreaks of MPBs, climate change, and the non-native white pine blister rust (Gibson et al., 2008; Tomback and Achuff, 2010). Great Basin bristlecone pine is of particular interest because it is one of the longest-lived organisms on Earth, reaching ages approaching 5,000 years, and one of the most highly fragmented high elevation conifer species (Ruiz-Olmo, J., Loy, A., Cianfrani, C., Yoxon, P., Yoxon, G., Silva, P. K. de, Roos, A., 2003). A better understanding of how MPBs locate and select hosts in high elevation systems will help us predict impacts and could allow development of tactics to manage MPBs in these



important, at-risk communities. Moreover, the discovery that MPBs avoid bristlecone VOCs helps shed light on the great longevity of bristlecone pines. It is likely that bristlecone pines possess additional defense mechanisms to MPBs (e.g. phloem defensive chemistry) and that the VOCs provide long-distance cues about host quality to beetles.

In conclusion, we show that female MPBs use VOCs in the initial location and selection between limber and bristlecone pines and that D-limonene plays a role in concert with other unidentified compounds. Such a role for VOCs in host location by MPBs is not surprising given VOCs represent a readily-available cue for foraging beetles, and that MPBs utilize a sophisticated VOC communication system to coordinate mass attacks once hosts are located (Safranyik and Carroll, 2007). These findings beg more questions. We examined foliar VOCs since they should represent the largest odor source, but what about VOCs emitted from boles (the portion of a tree attacked by beetles), do they differ from foliar VOCs and are they used by MPBs? Which compounds and ratio of compounds are used by MPBs to find limber pines and avoid bristlecone pines? Are there common similarities and differences between VOCs of hosts and non-hosts that MPBs could use as general rules when searching for hosts? How do VOCs change with host condition and does this affect beetle's choices? Finally, VOCs underlying mass attacks have been successfully used to manage MPBs (Progar et al., 2014) and the results presented here suggest that VOCs used in host location have been overlooked but might similarly be exploited for management of MPBs.

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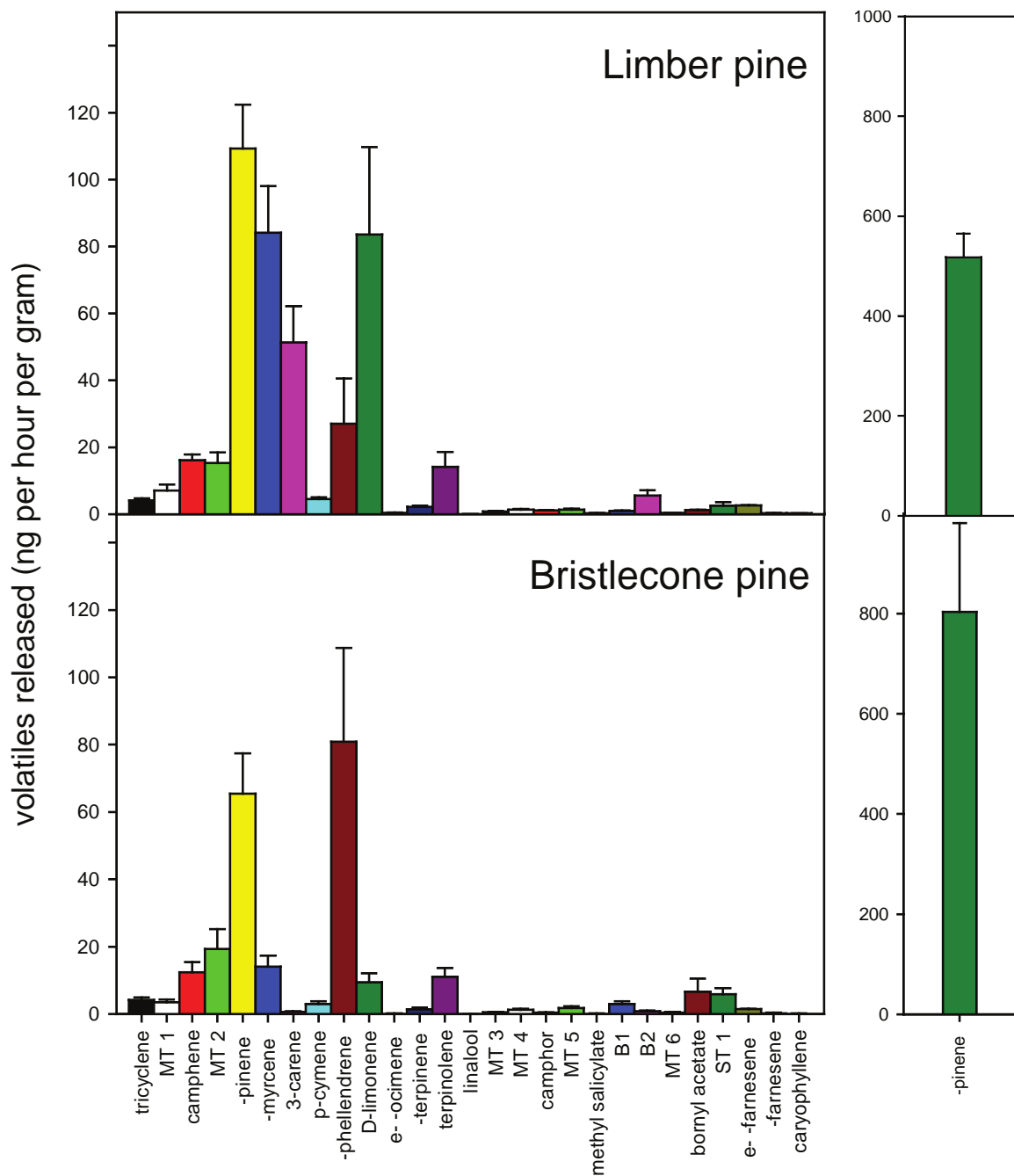
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## Supplemental



**Fig. S7.** Total volatiles emitted (ng per hour per gram  $n = 4$ ) by limber pine (*Pinus flexilis*) and Great Basin bristlecone pine (*Pinus longaeva*) clipped foliage used in Y-tube trials. Note different scale for  $\alpha$ -pinene.

## CHAPTER 6

### CONCLUSION

This dissertation explored the unique fuels composition and chemical response of Earth's oldest living tree species, the Great Basin bristlecone pine. I examined the chemical response of volatile organic compounds (VOCs) emitted from Great Basin bristlecone pine foliage and within foliage compounds in response to elevational gradients, and how tree VOCs cue mountain pine beetle host selection. This research is multidisciplinary and incorporates landscape ecology, disturbance ecology, resiliency, and disturbance response to evaluate the health and status of Great Basin bristlecone pine, a unique species with a limited distribution and a specialized niche. To understanding a long-lived species response to climate change, it is critical to understand how this slow-growing species may be influenced by the multiple of climatic fluctuations faced during their lifetimes. Some populations may be more vulnerable to climatic changes by virtue of their longevity, while others such as Great Basin bristlecone pine may use longevity as a buffer against climatic variability and a constant source of new seed. Researching this fragmented species is challenging because inventorying small patches is difficult and small changes in one stand can have large effects on the total population.

Below, I summarize three general findings emerging from this research. The greatest fire threat to Great Basin bristlecone pine growing near treeline is from fires ignited in the mixed conifer forests and shrublands below and progressing as crown fires into pure stands of Great Basin bristlecone pine during extreme wind conditions and exceptionally dry fuel moistures. However, current monitoring programs inadequately quantify the surface and canopy fuels of this system. The first finding (Chapter 2)



addresses the likely changes and amplification of ecological disturbances due to anthropogenic climate warming. I focus on the disturbance of wildland fire and alteration of fuels. Linear regression showed that all classes of fine woody debris (FWD) had an inverse relationship with elevation, yet coarse woody debris (CWD) remained constant across elevational transects (Gray and Jenkins, 2017). Fuel sampling in the pine fuel zone (the area directly beneath the tree crown) (Jenkins, 2011), helps describe the discontinuous fuels found at alpine treeline in Great Basin bristlecone pines. Accounting for these poorly studied fuels, lower crown base height (CBH) and foliar moisture, combined with warming temperatures due to climate change, and suggests an increase in fire potential at the Great Basin bristlecone pine treeline, threatening the oldest individuals of this iconic species. However, the likelihood of surface fire decreases with elevation, the potential for crown fire and spotting still exist. More research is needed to identify the wind speed and fuel moisture thresholds that could sustain crown fire propagation. New information about discontinuous fuels will aid in management of high elevation alpine treeline forests.

The second finding (Chapter 3) of this research addresses the physiological response of Great Basin bristlecone pine to climate warming-induced changes to environmental factors. The physiological response of VOC emissions and within-needle terpenoid concentrations provide a promising indicator of an individual trees response to environmental conditions. The blend of VOCs emitted from Great Basin bristlecone foliage proved to be an excellent predictor (82% accuracy) for the elevation of the tree. Additionally, air temperature, heat load index (potential direct radiation at a site), and relative humidity correlated well with emitted VOCs. The changes in the amounts of

individual compounds (e.g E- $\beta$ -ocimene,  $\alpha$ -terpinene,  $\beta$ -phellandrene) could provide insight into a tree's response to environmental stress. Several of the identified emitted compounds have been shown to interact with mountain pine beetle, provide relief from oxidative and thermal stresses, and to increase foliage flammability (Page et al., 2012). These changes, along environmental gradients (increased VOC at different elevations), occur in response to biotic or abiotic stressors. This improved understanding of Great Basin bristlecone pine response to elevational gradients (as a proxy for climate warming), aids in discovering potential susceptibility to threats like fire or mountain pine beetles. This research improves understanding of VOC emissions as a physiological tree response to environmental factors and helps inform land managers about forests threatened by native and non-native pests and pathogens, increased threat of fire, and changing distribution patterns from climate change.

The final chapter (Chapter 4), describes differences of emitted VOCs and how mountain pine beetle (a native pest to many *Pinus* species in North America) use these compounds to identify suitable tree hosts. The comparison of VOCs emitted from Great Basin bristlecone pine versus limber pine revealed several differences in compounds. The most dramatic difference were in 3-carene (70 times more abundant in limber pine),  $\beta$ -myrcene (4.8 times more abundant in limber pine), and D-limonene (4.6 times more abundant in limber pine)(Gray et al., 2015). My research showed that female mountain pine beetle use VOCs in the initial selection between limber pine, a preferred host, and are repelled by VOCs emitted from Great Basin bristlecone pine, a non-host. D-limonene, combined with other unidentified compounds, plays a role. A better understanding of how mountain pine beetles locate and select hosts in high elevation systems will help us

predict impacts of beetle attacks and could help develop tools to manage mountain pine beetle in these important communities.

### **Contributions and Broader Impacts**

This dissertation improves understanding of the resiliency and response of Great Basin bristlecone pine to disturbance. In addition to biodiversity's role as mitigation against changing environments and disturbances, there is increasing evidence that the collective role of rare species may be important for ecosystem services (Tschardt et al., 2005). The contribution of each rare species is usually small, but all species together may be of quantitative importance (Tschardt et al., 2005). My research and findings are useful for land managers to prioritize strategies to insure the health of Great Basin bristlecone pine, as well as to manage other rare, yet charismatic, species. Another contribution of this work is that I have researched a wide extent of Great Basin bristlecone pine populations. The majority of other Great Basin bristlecone pine research has been completed at only three sites: the Ancient Bristlecone Pine Forest in California, Wheeler Peak in Great Basin National Park, and the Spring Mountains National Recreation Area. While Great Basin bristlecone pine is a very long lived species and individuals are quite resilient to climate variability, the species as a whole is often overlooked by land managers, who focus instead on protecting high profile, ancient trees located in national parks and near visitor centers, or who focus on more common timber producing species. The fragmentation and isolation of distinct populations of Great Basin bristlecone pine affect gene flow, which potentially reduces resiliency to large landscape disturbances. It also makes coordinated management difficult. To adequately manage this

species into the future, we must examine Great Basin bristlecone pine's intrinsic rareness on the landscape, how warming temperatures and invasive species might change disturbance regimes and frequency, and focus on management strategies that provide for future groves. Complex interactions between climate and disturbance regimes influence current and future forest communities, however it is challenging to develop management strategies which maintain resistance and resilience in forest communities.

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## CURRICULUM VITAE

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**RESEARCH INTERESTS**


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Forest resources management, Forest Ecology, Disturbance Ecology, Remote Sensing/GIS, and quantitative analysis. My research studies the interaction of agents of disturbance in conifer forests over large spatial and long temporal scales, focusing on the high elevation systems of the Great Basin bristlecone pine.

**EDUCATION**


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**Utah State University**, Ph.D. in Ecology (projected 2017)  
 Dissertation: “Impact of Climate Variability on the Frequency and Severity of Ecological Disturbances in Great Basin Bristlecone Pine Sky Islands” (Dr. Michael J. Jenkins, advisor)

**San Diego State University**, M.A. in Geography 1995  
 Thesis: “Predicting the Location of Riparian Vegetation using Landsat TM and Digital Terrain data in the Cleveland National Forest” (Dr. Janet Franklin, advisor)  
 ▪ Emphasis: Geographic Information Systems and Remote Sensing

**University of California, Santa Barbara**, B.A. in Geography and Environmental Studies 1992  
 Thesis: “Ionic Pulse in Sierra Snowpack” (Dr. Robert Davis, advisor)  
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**WORK EXPERIENCE**


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2011 – present	Research Assistant, Department of Wildland Studies, Utah State University. Logan, Utah
2014, 2016	Lecturer, Utah State University. Logan, Utah
2005 – 2011	Research Analyst – Geographic Information Systems, California Department of Parks and Recreation, Sierra District. Tahoe City, CA
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- 1997 – 2001 GIS Analyst/Project Manager, Pacific Meridian Resources, USFS Remote Sensing Lab/CAL FIRE, Fire and Resource Assessment Program, Sacramento CA
- 1996 – 1997 Project Manager, Department of Geography, San Diego State University, San Diego, CA
- 1995 – 1996 Assistant Project Manager, Department of Geography, San Diego State University, San Diego, CA
- 1993 – 1995 Graduate Research Assistant, Department of Geography, San Diego State University, San Diego, CA
- 1993 Project Assistant, SDSU-NCR Validation Center, San Diego State University, San Diego, CA
- 1993 City Planning Intern, Upland City Hall, Upland, CA
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## **PUBLICATIONS**

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### **Peer Reviewed Journal Articles**

Gray CA, and Jenkins, MJ., (2017) Climate warming alters fuels across elevational gradients in Great Basin bristlecone pine-dominated sky island forests. Accepted (*Forest Ecology and Management*)

T Beach, SE Null, CA Gray (2016) An affordable method of thermal infrared remote sensing of wadeable rivers using a weather balloon. *Journal of Undergraduate Research and Scholarly Excellence* 7 (I), 26-31

Gray CA, Runyon JB, Jenkins MJ, Giunta AD (2015) Mountain Pine Beetles Use Volatile Cues to Locate Host Limber Pine and Avoid Non-Host Great Basin Bristlecone Pine. *PLoS ONE* 10(9): e0135752. doi:10.1371/journal.pone.0135752

Franklin, Janet, Paul McCullough, and Curtis Gray. "Terrain variables used for predictive mapping of vegetation communities in Southern California." *Terrain analysis: principles and applications* (2000): 331-353.

### **Near Submission**

Gray CA, Runyon JB, Jenkins, Using Multivariate Ordination to Characterize Great Basin Bristlecone Pine Volatiles along Environmental Gradients to Assess Response to Climate Change.

### **Other Publications**

Teich, M., Schneebeli M., Bebi, P., Giunta A.D., Gray C.A., Jenkins M.J. 2016. Effects of Bark Beetle Attacks on Snowpack and Snow Avalanche Hazard. *Proceedings International Snow Science Workshop*, October 2016.

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- Franklin, J., P. McCullough, C. Gray and D. Simons. Terrain variables used for predictive mapping of vegetation communities in Southern California, Terrain Analysis Workshop, Third International Conference/Workshop on Integrating GIS and Environmental Modeling, Santa Fe, New Mexico, January 21-25, 1996.

## **RESEARCH GRANTS AND FUNDING**

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USFS EM Project INT-EM-F-537 10-02, the Ecology Center at Utah State University, the Utah Agricultural Experiment Station (UAES), 538 and a USDA National Needs Graduate Fellowship Competitive NIFA Grant.

## **TEACHING**

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### **Courses Taught at Utah State University (since 2011)**

*Forest Vegetation Disturbance Ecology and Management* – Wildland Studies 5710, 2014, 2016

*Avalanche and Snow Dynamics* (Teaching Assistant for Dr. Michael Jenkins) –Wildland Studies 5460, 2011-present

*Forest Vegetation Disturbance Ecology and Management* (Teaching Assistant for Dr. Michael Jenkins) –Wildland Studies 5710, 2013

*Wildland Ecosystems* (Teaching Assistant for Dr. R. Douglas Ramsey) –Wildland Studies 3800, 2013

*Assessment and Synthesis in Natural Resource Science* (Teaching Assistant for Dr. Fee Busby and Dr. R. Douglas Ramsey) –Wildland Studies 4910, 2013

## **PROFESSIONAL AWARDS AND HONORS**

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- Governor’s Emergency Response award for Witch/Harris fires 2007.

## **OTHER PRESENTATIONS**

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Gray, CA, Runyon JB, Jenkins, MJ. Characterizing Great Basin Bristlecone Pine Chemistry along Environmental Gradients to Assess Response to Climate Change, Restoring the West 2016.

Gray, CA. The Impact of Climate Variability on the Frequency and Severity of Ecological Disturbances in Great Basin Bristlecone Pine in Sky Islands. Oral Presentation. Intermountain Society of American Foresters Annual Spring Meeting, Logan UT. April 2014

Gray, CA, Runyon JB, Jenkins, MJ. Exposure to wildfire smoke alters tree chemistry of high elevation conifers. Poster Presentation. Restoring the West: Restoration and Fire in the Interior West. October 2015. Logan, UT.

Gray, CA, Runyon JB, Jenkins, MJ. Characterizing Great Basin bristlecone pine tree chemistry. Poster Presentation. International Union of Forest Research Organizations World Congress: Sustaining Forest, Sustaining People, October, 2014. Salt Lake City, UT.

Jenkins, MJ, Gray, CA, Runyon JB, Alexander, ME, Page, WG, Toone, C. Evaluation Monitoring Funded Projects Explain Relationships Between Bark Beetles, Fuels and Fire



Behavior. Poster Presentation. USDA Forest Service Environmental Monitoring Meeting April 2013.

Jenkins, MJ, Alexander, ME, Page WG, Gray, CA, Toone, C. Evaluation Monitoring: Monitoring on the Margin Funded Projects Explain Relationships Between Bark Beetles, Fuels and Fire Behavior. Poster Presentation. USDA Forest Service Environmental Monitoring Meeting April 2012.

### **VOLUNTEER SERVICE**

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- Nordic United, 2012 - present
- Alpine Meadows National Ski Patrol, 2010 - 2017
- Royal Gorge National Ski Patrol, 2008 – 2011
- Truckee River Aquatic Monitoring Program, 2008 – 2010

### **SKILLS AND QUALIFICATIONS**

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- Strong working knowledge of the UNIX environment (including shell programming and administration).
- GIS/IP experience: ArcMap (suite of ESRI products), Arc/Info (including AML), Arcview, AVENUE, FARSITE, Erdas, Imagine, Image Processing Workbench (IPW), and IDRISI.
- Experience with cartographic and statistical software on Macintosh and Windows (SPLUS and HTML).
- System administration skills including backups, printer queues, account administration, and debugging network problems.
- Basic computing: Microsoft Word, Excel, PowerPoint
- Programming: Experience with Visual Basic, R statistical program, and Python programming languages
- Languages: native English, workable Spanish
- Medical training: EMT-Basic, Wilderness First Responder, Outdoor Emergency Technician, Swiftwater Rescue Tech – I
- Field Techniques: FIA plot installation, Volatile Organic compound collection, temperature monitoring, longitudinal and cross-sectional profiling, fish snorkel surveying.

### **SCHOLARLY ASSOCIATION MEMBERSHIP**

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- Society of American Foresters (SAF)
- American Avalanche Association (AAA)