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ARTICLE

Essential oil content of *Rhododendron tomentosum* responds strongly to manipulation of ecosystem resources in Arctic Alaska[1]

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Abstract: The essential oils of the widely distributed boreal/Arctic dwarf shrub, *Rhododendron tomentosum* ssp. *subarcticum* (Harmaja) G.D. Wallace, have important ecological, cultural, medicinal, and commercial roles. To understand the relationship between resource limitation and essential oil content of this species we measured the amount and diversity of terpenes from shoots of plants exposed to a 14-year ecosystem resource manipulation experiment in Arctic Alaska. Treatments tested interactive effects of nitrogen (N) and phosphorus (P) addition, warming and N + P fertilization, and shading and N + P fertilization. The controls and NP fertilization had the highest essential oil content, whereas shading and P addition had less than 20% of the control content. Warming reduced essential oil content to <65% of that of the controls. Essential oil components varied greatly among the treatments, with significant differences in the expression of specific essential oil components. Large changes in plant community composition and ecosystem structure in response to treatments likely played a large role in the response of *R. tomentosum*. Our data suggest that resource changes in response to climate warming and its secondary effects on light and nutrient availability have the potential to change the profiles of essential oils in *R. tomentosum*, with important ecological and cultural impacts.

Key words: Toolik Field Station, Arctic LTER, shade, warming, nitrogen, phosphorus, fertilization, terpenes.

Résumé : Les huiles essentielles de *Rhododendron tomentosum* ssp. *subarcticum* (Harmaja) G.D. Wallace, un arbuste nain boréal/arctique largement répandu, exercent des fonctions écologiques, culturelles, médicinales et commerciales importantes. Pour comprendre la relation entre la limitation des ressources et la teneur en huile essentielle de cette espèce, les auteurs ont mesuré la quantité et la diversité des terpènes provenant de pousses de plants soumis à une expérience de manipulation des ressources de l'écosystème dans l'Alaska arctique d'une durée de 14 ans. Les effets interactifs de l'ajout d'azote (N) et de phosphore (P), du réchauffement et de la fertilisation N + P, ainsi que de l'ombrage et de la fertilisation N + P ont été examinés. Les témoins et les arbustes soumis à une fertilisation N + P montraient la plus haute teneur en huile essentielle, tandis que l'ombrage et l'ajout de P produisaient des arbustes ayant moins de 20 % de la teneur des témoins. Le réchauffement réduisait la teneur en huiles essentielles à <65 % de celle des témoins. Les composants des huiles essentielles variaient considérablement en fonction des traitements, avec des

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différences significatives dans l'expression des composants spécifiques des huiles essentielles. Les changements importants dans la composition de la communauté végétale et la structure de l'écosystème en réponse aux traitements ont probablement joué un rôle important dans la réponse de *R. tomentosum*. Ces données suggèrent que les changements en matière de ressources en réponse au réchauffement climatique et ses effets secondaires sur la disponibilité de la lumière et des nutriments ont le potentiel de modifier les profils d'huiles essentielles chez *R. tomentosum*, avec des impacts écologiques et culturels importants. [Traduit par la Rédaction]

Mots-clés : station expérimentale de Toolik, site de recherche Arctic LTER, ombre, réchauffement, azote, phosphore, fertilisation, terpènes.

Introduction

The highly-cited resource availability hypothesis predicts that allocation to plant defensive compounds is a function of available resources, with resource availability inversely related to allocation to defense (Coley et al. 1985; Endara and Coley 2011). As the climate changes, resource availability, and in response, ecosystem processes, are expected to change substantially (Shaver et al. 2000; Parmesan and Yohe 2003). Nowhere are these changes expected to be stronger than in the Arctic; temperatures in the Arctic have risen 2.4 times faster than the average of the Northern Hemisphere, consistent with general circulation model predictions (AMAP 2019). These temperature changes and their consequences will potentially alter the quantity and profiles of plant defense compounds that in turn may affect the ecological interactions among species as well as human subsistence and commercial use of important secondary metabolites. Despite the potential for large changes, few studies have experimentally tested the potential effects of resource variation on secondary compound production by Arctic and alpine species (Nybakken et al. 2008).

The resource availability or growth rate hypothesis primarily addresses interspecific variation in allocation to defensive compounds, although it has also been applied to intraspecific variation (Hahn and Maron 2016). Variation within species has been evaluated in the context of both genetic (ecotypic) or within-plant differences in response to variation in external factors and resources (Koricheva et al. 1998; Hamilton et al. 2000; Yang et al. 2018). Hahn and Maron (2016) proposed a predictive framework for studies of genetic differences in intraspecific production of defensive compounds, but changes resulting from differences in resource allocation are poorly understood. Furthermore, defensive compounds may be produced constitutively or induced by damage (Agrawal 2011). The dominant factor(s) driving intraspecific variation in defensive compounds may be species dependent. In *Pastinaca sativa,* induced damage was the strongest driver of furanocoumarin levels, with the weakest drivers being light and nutrient variation (Zangerl 1986). By contrast, the tannin and total phenolic composition of *Betula pubescens* ssp. *tortuosa* was most affected by fertilization and shade, followed by damage (Ruohomaki et al. 1996). Generally, species with low constitutive defenses should have high inducible defenses (Endara and Coley 2011). However, some recent studies have found that plants of the same species tend to have higher constitutive defenses in high resource environments than in low resource environments because of high herbivory pressure in resource-rich environments (Hernán et al. 2019). These species-specific responses complicate efforts to understand community responses to resource changes.

Here, we experimentally addressed the role of resource limitation on intraspecific variation of essential oil content in the tundra and northern peatland aromatic plant, *Rhododendron tomentosum* ssp. *subarcticum* (Harmaja) G.D. Wallace (Ericaceae: synonymous with *Ledum palustre* ssp. *decumbens* (Ait.) Hultén and commonly called marsh rosemary or marsh Labrador Tea). Ecologically, *R. tomentosum* ssp. *subarcticum* is a slow-growing, dwarf evergreen shrub species with a circumpolar distribution, representing up to 50% of vegetative cover in wet shrub tundra and as a dominant understory shrub in boreal forest (Bliss and Matveyeva 1992). *Rhododendron tomentosum* ssp. *subarcticum* is found primarily in low nutrient habitats; in enriched nutrient environments, it is overtopped by faster growing species (Chapin and Shaver 1996). In low nutrient sites, the evergreen leaves may persist up to five years (Shaver 1981). Multiple terpenoid compounds have been identified in the essential oil from *R. tomentosum* (Tattje and Bos 1981) and the closely related boreal species, *Rhododendron groenlandicum* (Belleau and Collin 1993). Essential oil production in *Rhododendron* is strongly affected by seasonality (Prudhomme 1983; Butkienė and Mockute 2011) and shoot age (Butkienė et al. 2008). Terpenoid compounds are known antiherbivore and allelopathic agents; Reichardt et al. (1990) identified the terpenoid germacrone as the primary defensive compound against snowshoe hare herbivory. *Rhododendron*-derived compounds have been identified as allelopathic in black spruce forest (Inderjit and Mallik (1997) and affecting nitrogen cycling in boreal hardwood forests (Castells et al. 2003).

Economically, the shrub is an important food and medicinal source for circumpolar native peoples because of the high content of bioactive essential oils (Dampc and Luczkiewicz 2013). Native people in Alaska use the plant primarily as a beverage flavoring, seasoning, or chewed raw. Extracts of *R. tomentosum* was used a bittering agent in beer before the widespread adoption of hops (Grieve 1971). However, according to Hultén (1968), *R. tomentosum* ssp. *subarcticum* is mildly toxic because it contains the poisonous sesquiterpene, ledol. The essential oil of the shrub has been historically used in the treatment of puncture wounds, spider bites, coughs, colds, bronchial, and pulmonary disorders by both Indigenous and non-Indigenous peoples (Dampc and Luczkiewicz 2013), and in some communities Labrador tea is the most commonly used medicinal plant (Walker 1984; Kari 1991). Recent studies have found antimicrobial, antioxidant (Kim and Nam 2006), analgesic (Zhang et al. 2010), anti-inflammatory (Baananou et al. 2015), and insecticidal (Benelli et al. 2020) properties of essential oils from *Rhododendron*. Many studies have reported the site-specific composition of essential oils of varieties of *Rhododendron* throughout the higher latitudes of the northern hemisphere (De-xiu et al. 1987; Kim and Nam 2006; Raal et al. 2014; Judžentienė et al. 2012; Belousova et al. 1990), with over 90 different compounds reported (Judžentienė et al. 2012).

Despite the importance of *R. tomentosum* ssp. *subarcticum*, the effects of resource availability on essential oil production in this species have not been tested. Primarily found on cold, slowly decomposing soils, *R. tomentosum* ssp. *subarcticum* growth is generally limited by temperatures and nutrients. As the climate warms and soil decomposition rates increase, both temperature and nutrient limitations will be reduced (Suzuki and Kudo 1997). The expression of secondary compounds in *R. tomentosum* will almost certainly shift in response to changes in resources as the climate warms, with potentially important ecological consequences.

Our objective was to understand how resource availability changes may affect essential oil content in *R. tomentosum*. Given their low resource environment, we would expect these plants to have high constitutive defense; if resources are scarce then investment in protective compounds are advantageous. To meet this objective we assessed the essential oil components of *R. tomentosum* ssp. *subarcticum* in response to 14 years of a long-term resource limitation experiment in northern Alaska that manipulated light, temperature, and soil nutrients, and their interactions, to understand the factors limiting production in tundra and provide insight into changes that may occur in response to climate warming (Bret-Harte et al. 2001). To understand the physiological background for responses, we assessed leaf nutrients and photosynthetic properties along with the essential oil analysis.

We consider only constitutive defense with no attempt to trigger inducible defenses. Our approach was to quantify the total amount and diversity of terpenes in the essential oil present in response to the treatments without focusing on the specific identities of the compounds encountered. We concentrated on essential oils because of their ecological, subsistence, and commercial importance in the study species. We tested the hypothesis that treatments or treatment combinations that increase nutrient availability or reduce carbon gain would decrease constitutive defense as indicated by essential oil content and diversity. Fertilizer additions with nitrogen are expected to decrease carbon-based defense compounds (De Long et al 2016) such as terpenes by increasing nitrogen content, and shading will decrease carbon content and increase nitrogen content because of lower nutrient demand under lower carbon availability. Given that the plants were part of a long-term experiment, and community composition changed in response to treatments, interpretation of the effects of treatments on *R. tomentosum* ssp. *subarcticum* must consider the potential effects of these community changes on essential oil production. The results of this study provide the first indications as to how essential oil content of this important species changes in direct response to long-term alterations of a suite of key resources as well as in response to the ecosystem changes that have taken place in response to the original treatments after 14 years.

Methods

Study site

The study site, Toolik Field Station, is located in the northern foothills of the Brooks Range in northern Alaska (68°38′N, 149°38′W) at an elevation of 720 m above sea level. The dominant vegetation in the region is moist acidic tussock tundra with *Eriophorum vaginatum* L., *Rhododendron tomentosum* ssp. *subarcticum*, and *Betula nana* L. as co-dominant species. The growing season is typically mid-May to late August and has a mean temperature of 7 °C (Arctic Long Term Ecological Research (LTER) project, https://arc-lter.ecosystems.mbl.edu). Previous fertilization studies at the site indicate that nitrogen is the dominant limiting nutrient (Chapin et al. 1995), although a recent study implicated limitation by both nitrogen and phosphorus (McLaren and Buckeridge 2019).

Treatments

Rhododendron tomentosum ssp. *subarcticum* tissue was collected from the Arctic LTER terrestrial experimental plots at Toolik Lake, Alaska, USA. These plots are a series of factorial resource manipulations started in 1988 (Bret-Harte et al. 2001) that were patterned after ongoing earlier prototype studies conducted at the same site to evaluate controls on productivity in tundra ecosystems with the aim to understand potential responses to climate change (Chapin et al. 1995; Chapin and Shaver 1996). The LTER study included four replicate blocks with each block consisting of ten 5 × 20 m^2 plots separated by a 2 m buffer strip (Supplementary Figure S1[2]). The ten plots were randomly assigned to the following treatments: yearly nitrogen addition (N), year phosphorus addition (P), yearly nitrogen + phosphorus addition (NP), a shadehouse plot with shade only (SC; S added shade; C, control for nutrients) and shade + nitrogen + phosphorus (SNP), a greenhouse plot with warming only (GC; G greenhouse warming; C, control for nutrients) and warming + nitrogen + phosphorus (GNP), two overall controls (C), two animal exclusion plots not included in this study, and an extra plot. The plots modifying the light and temperature environments (shading and warming) had two structures (subplots, 2.5 × 5 m) within a 5 × 20 m plot, with

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a structure without added nutrients 2 m upslope of a structure with added nutrients. The warming treatments simulated predicted increased temperatures, the shade treatments simulated increased cloudiness or overtopping by shrubs or trees, and the nutrient additions simulated the effects of increased soil thaw and the release of nutrients via decomposition. The design was not a full factorial but rather three two-way factorial experiments, Shade by NP fertilization, Temperature by NP fertilization, and Nitrogen by Phosphorus fertilization. Attenuated light was achieved using optically neutral shade cloth that removed approximately 50% of the incoming radiation. Temperature was increased using transparent greenhouse plastic (0.15 mm thickness). Both light attenuation and increased temperature treatments used wooden frames upon which the treatment material was stretched. A potential artifact of the greenhouses is reduced photosynthetically active radiation loads and UV by filtering by the greenhouse plastic (Hansen et al. 2006) and condensation (Chapin et al. 1995). The wood frames had a gable roof 65 cm above the ground surface along the edges and 1.3 m tall in the center. Both warming and shade wooden frames were permanently installed, with the treatment material stretched over the frames following snow melt each spring and removed before snow accumulation in the fall. Nutrient addition consisted of the application of 10 $g{\cdot}m^{-2}$ nitrogen as NH_4NO_3 and 5 $g{\cdot}m^{-2}$ phosphorus as P_2O_5, amounts well above the annual demand for these nutrients by the plot vegetation. Further information regarding these plots is available in Bret-Harte et al. (2001). Unlike the prototype experiment, no comprehensive publications comparing productivity and biomass harvest data across treatments are available for the plots used in the current study. Consequently, for such comparisons we refer mostly to published results from the prototype experiment (Chapin et al. 1995, Chapin and Shaver 1996, Shaver et al. 2001).

Sample collection and physiological measurements

We sampled five individual shoots consisting of a terminal meristem and the subtending leaves of the current and previous years' growth in each replicate of each treatment ($n = 4$ blocks). At this site leaves of *R. tomentosum* ssp. *subarcticum* generally persist one to two years, with the previous years' leaves mostly senescing shortly after new leaves are produced. In some shaded treatments, *R. tomentosum* ssp. *subarcticum* was no longer present, reducing the sample size below the number of blocks (4). Each collection was random within a 3 m^2 area inside the area of each replicate plot. Each individual was measured for maximum photosynthesis, yield of fluorescence, and dark-adapted fluorescence (F_v/F_m) to assess the physiological status of each plant within the treatments. Maximum photosynthesis (A_{max}) was measured using a LI-6400 photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA) at a photon flux density of 1500 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and CO_2 concentration at 380 $\mu mol{\cdot}mol^{-1}$. The entire terminal shoot was placed in the LI-6400 with a 2×3 cm chamber. Leaf areas were estimated from length-to-area regressions for individual leaves. Fluorescence yield determined overall photochemical quantum yield or yield of energy conversion under ambient light. Dark-adapted fluorescence determined potential quantum efficiency, using the ratio of variable fluorescence (F_v) to maximum fluorescence (F_m) of samples that were darkened by leaf clips for at least 15 min prior to measurement. Fluorescence measurements were made using an OS-5 fluorometer (Opti-Sciences, Tyngsboro, Massachusetts, USA).

After physiological measurements were completed for all treatments, the marked aerial parts were collected and placed on ice until they were frozen in a −20 °C freezer and then shipped frozen back to the laboratory where they were stored frozen at −40 °C until analysis. All tissue collections for essential oil analysis were on the same day at near peak biomass (18 July) within a 2 h period to eliminate variation caused by either diurnal (Zhang et al. 2017) or seasonal effects (Butkiene and Mockute 2011).

Leaf nutrients

For nutrient analysis, we collected the nearest shoots on the same plant as those collected for essential oil determination and dried the leaves to determine foliar carbon: nitrogen and percent phosphorus for each treatment. The C:N ratio in dried leaf samples was measured using an elemental analyzer (Carlo Erba, Valencia, California, USA). Percent phosphorus was measured using a molybdate antimony volumetric technique (Fourqurean and Zieman 2002). Samples were weighed, ashed at 800 °C for 6 h in a muffle furnace, acidified, and reacted with ammonium molybdate and antimony. The resulting solution was measured by spectrophotometer (Shimadzu, Columbia, Maryland, USA).

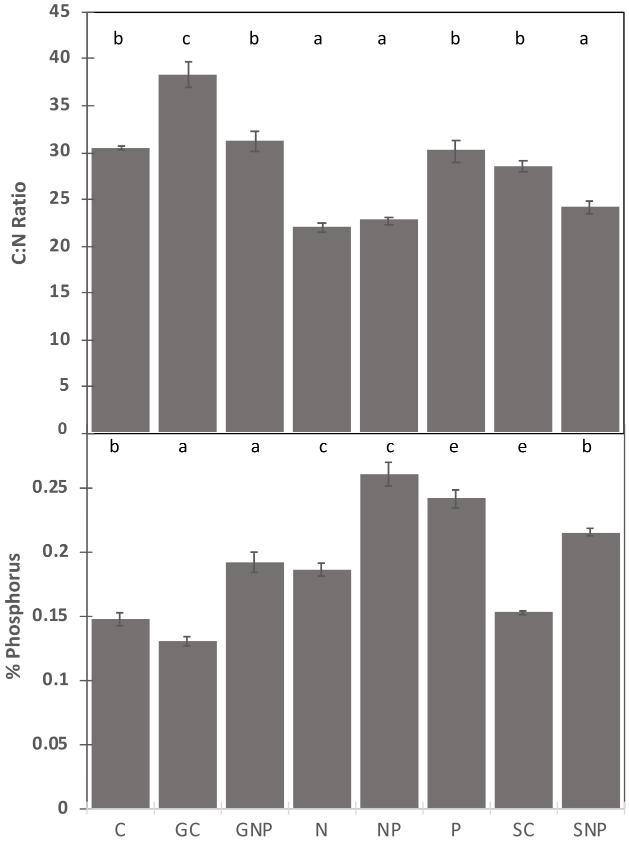
Essential oil analysis

Essential oil analysis was conducted using a static headspace sampling technique. The goal was to determine total amount of essential oil and quantities of the different components but not to chemically identify all of the components. To determine the number and quantity of the different components, 0.23 g of leaves and apical growth stems were placed in a 10 mL headspace autosampler vial. No effort was placed on selecting a specific leaf age or quality as the physiological measurements were taken on the entire shoot. Apical growth stems were included in the analysis because preliminary assays determined the presence of the essential-oil-producing hairs on the newest stems. The resulting samples contained a mixture of both new and old leaves representative of the plant at the time of collection along with the supporting stems. These samples were heated to 80 °C for 5 min to volatilize essential oil components and stabilize the temperature. A 25 μL sample of headspace was extracted and analyzed using a gas chromatograph with an attached mass spectrometer (Hewlett Packard 5973 MSD, 0.25 μm i.d., 30 m, DB-5 capillary column, Santa Clara, California, USA). This headspace technique allows for rapid identification and subsequent quantification of the major components in the essential oil while also minimizing the adulteration of the compounds known to occur in other extraction methods. Quantity of components was measured using peak area analysis. The compounds with a detectable peak area were qualitatively determined and the most prevalent compounds were measured quantitatively to determine variation among samples. These compounds were α-pinene, β-pinene, cymene, camphene, γ-terpenol, unknown monoterpene 1, α-phellandrene, allocymene, unknown sesquiterpene 2, sabinene, unknown sesquiterpene 3, and terpinene.

Statistical analysis

To provide a general overview of the effects of all experimental treatments in one analysis, leaf nutrients and physiological parameters were compared with one-way analyses of variance (ANOVAs) and Tukey's post hoc tests. However, this experiment was specifically designed for three separate two-way ANOVAs to determine the interactive effects of addition of N, P and N + P, shading, and warming on variation in leaf nutrients, physiological parameters, and quantity and diversity of terpenes in the essential oils as described previously in the "Treatments" section. In those ANOVAs, block was treated as a random factor. Where appropriate, variables were transformed to improve homogeneity of variances. Relationships between physiological parameters and total and number of terpenes were tested using Pearson's Correlation analysis. Finally, partial correlation analysis was used to clarify the relationships between nutrients and essential oil components. Non-metric Multi-Dimensional Scaling (nMDS) analysis was performed using the presence of the different terpenes and their abundance to examine the response of all compounds for patterns in response to treatments, but no coherent patterns were found (data not shown).

Fig. 1. Carbon to Nitrogen (C:N) ratios and percent Phosphorus in dried leaves of experimentally manipulated *Rhododendron tomentosum* ssp. *subarcticum* by treatment (C, Control; GC, Warming; GNP, Warming + Nitrogen + Phosphorus; N, Nitrogen; NP, Nitrogen + Phosphorus; P, Phosphorus; SC, Shade; SNP, Shade + Nitrogen + Phosphorus). Values are mean ± SE. Different lowercase letters above a bar indicate that the values are significantly different at $p < 0.05$ using comparison of means within a one-way analysis of variance (ANOVA) and Tukey's post hoc tests. $N = 4$ per treatment. All measurements were arcsine square root transformed prior to analysis.



The statistical package SPSS (versions 21 and 27 SPSS, Inc, Chicago, Illinois, USA) was used for all analysis with the exception of the nMDS, which was conducted in the statistical environment R using the vegan module (R Core Team, Vienna, Austria, https://www.r-project.org/).

Results

Leaf nutrients

The C:N ratio was lowest in the Nitrogen addition (N) and the highest in the GC treatment (Fig. 1). All nitrogen addition treatments had a lower C:N than the other treatments except for GNP, which was higher than those of SC, P, and C. Leaf phosphorus was lowest in the control treatments (C, GC, and SC), with the lowest value in GC (Fig. 1). Warming + nitrogen + phosphorus (GNP) resulted in lower percent phosphorus in the leaves compared with any other phosphorus additions (SNP, P, and NP). The highest P values were found in the NP and P treatments. Two-way ANOVA for effects of light revealed that light did not significantly affect C:N ratio or P content, whereas nutrient addition affected

Table 1. Results for leaf Carbon to Nitrogen (C:N) ratios and percent Phosphorus separated into three two-way analyses of variance (ANOVAs) to analyze for the effects of: (1) Light (shading), Nutrients (N + P addition), and the interaction of Light and Nutrients; (2) Temperature (greenhouse warming), Nutrients, and the interaction of Temperature and Nutrients; and (3) Nitrogen addition, Phosphorus addition and the interaction of both Nitrogen and Phosphorus addition for *Rhododendron tomentosum* ssp. *subarcticum.* All measurements were arcsine square root transformed prior to analysis. *p* values < 0.05 are highlighted in bold.

		C:N ratio		Phosphorus	
Effect	df	*F*	*p*	*F*	*p*
Light	1	0.049	0.850	5.64	0.141
Nutrient	1	21.7	**0.024**	19.9	**0.021**
Light*Nutrient	1	0.851	0.526	394.1	**0.032**
Temperature	1	22.7	**0.018**	9.54	0.054
Nutrient	1	11.4	**0.043**	33.0	**0.010**
Temperature*Nutrient	1	0.061	0.821	5.91	0.093
Nitrogen	1	58.7	**0.005**	23.2	**0.017**
Phosphorus	1	0.021	0.894	14.5	**0.032**
Nitrogen*Phosphorus	1	0.142	0.731	1.23	0.348

Table 2. Average maximum photosynthetic rate, A_{max}, (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), variable to maximum dark-adapted fluorescence F_v/F_m (unitless), fluorescence yield (unitless), and shoot leaf area (mm^2) of *Rhododendron tomentosum* ssp. *subarcticum* in response to treatments: C, Control; GC, Warming; GNP, Warming + Nitrogen + Phosphorus; N, Nitrogen; P, Phosphorus; NP, Nitrogen + Phosphorus; SC, Shade control; SNP, Shade + Nitrogen + Phosphorus. Values are mean ± SE. Within a column, means with the same lowercase letter are not significantly different at $p > 0.05$ using comparison of means within a one-way analysis of variance (ANOVA) and Tukey's post hoc test. $N = 4$ per treatment.

Treatment	A_{max}	F_v/F_m	Fluorescence yield	Leaf area
C	1.6 ± 0.3a	0.715 ± 0.010abc	0.498 ± 0.030b	107.6 ± 12.3ab
GC	2.8 ± 0.3b	0.729 ± 0.011abc	0.483 ± 0.035b	153.7 ± 19.0b
GNP	2.9 ± 0.4b	0.711 ± 0.010ab	0.406 ± 0.030ab	134.9 ± 22.6ab
N	2.2 ± 0.4b	0.701 ± 0.010ab	0.443 ± 0.030ab	140.5 ± 10.8ab
NP	1.8 ± 0.3b	0.728 ± 0.01abc	0.456 ± 0.035ab	139.6 ± 18.0ab
P	1.7 ± 0.3b	0.690 ± 0.010a	0.324 ± 0.030a	116.9 ± 13.8ab
SC	1.3 ± 0.4a	0.748 ± 0.020bc	0.447 ± 0.063ab	87.7 ± 14.1a
SNP	2.1 ± 0.5b	0.764 ± 0.020c	0.534 ± 0.063b	166.2 ± 24.8b

both (Table 1). The interaction between shading and nutrient addition was significant for P content. Two-way analysis of warming and increased nutrients showed significant increase in C:N ratio with warming and significant effects of fertilization on both C:N ratio and P content (Table 1). The test of the interactive effects of N and P addition separately and combined showed N addition decreased C:N ratio and increased P content, whereas addition of P alone increased P content only (Table 1). The interaction between N and P addition was not significant.

Leaf physiology and leaf area

Maximum photosynthesis (A_{max}) differed among the treatments with SC reducing photosynthetic potential to less than half that of the GNP (Table 2). The maximum in the SNP was nearly 60% greater than that in the SC, and in every instance, the mean A_{max} of the nutrient treatment was higher than that treatment's control treatment (for example, SC is lower than SNP, C is lower than NP, Table 2). The effect of reduced light on A_{max} was not quite

Table 3. Two-way analysis of variance (ANOVA) results for physiological measurements, average maximum photosynthetic rate, A_{max}, (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), variable to maximum dark-adapted fluorescence F_v/F_m (unitless), fluorescence yield (unitless), and shoot leaf area (mm^2) of *Rhododendron tomentosum* ssp. *subarcticum* separated to analyze the effect of Light, Nutrient, and the interaction of Light and Nutrient, Temperature, Nutrient, and the interaction of Temperature and Nutrient, and Nitrogen addition, Phosphorus addition, and the interaction of both Nitrogen and Phosphorus. *p* values < 0.05 are highlighted in bold.

		A_{max}		F_v/F_m		Fluorescence yield		Leaf area	
Effect	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Light	1	3.51	0.066	1.83	0.184	6.07	**0.018**	0.036	0.851
Nutrient	1	12.51	**0.001**	0.382	0.540	1.09	0.303	9.45	**0.003**
Light*Nutrient	1	2.60	0.112	1.01	0.322	0.477	0.494	1.68	0.200
Temperature	1	6.14	**0.016**	0.094	0.760	1.12	0.294	0.046	0.831
Nutrient	1	1.08	0.303	0.013	0.908	1.21	0.276	0.328	0.568
Temperature*Nutrient	1	0.543	0.464	0.191	0.664	0.372	0.544	5.50	**0.022**
Nitrogen	1	0.371	0.544	0.991	0.323	2.32	0.132	0.451	0.504
Phosphorus	1	3.45	0.067	0.111	0.740	4.48	**0.038**	2.05	0.157
Nitrogen*Phosphorus	1	1.57	0.215	4.56	**0.036**	12.4	**0.001**	0.675	0.414

significant, but NP addition strongly increased A_{max} when shaded, with no interaction (Table 3). In the temperature by nutrient addition analysis, increased temperature resulted in a significantly higher A_{max} (df = 1, *F* = 6.13, *p* = 0.016) without a nutrient effect or interaction. The effects of N and P addition separately were not significant with no interaction, though the P addition was close to significance. The ratio between variable and maximum fluorescence (F_v/F_m) for dark-adapted leaves was not significantly affected by the increased temperature or reduced light treatments (Table 2) but was affected by the interaction of nitrogen and phosphorus additions (df = 1, *F* = 4.563, *p* = 0.036, Table 3). The lowest F_v/F_m occurred with the addition of phosphorus alone (Table 2). For fluorescence yield, the addition of phosphorus alone resulted in a significantly lower yield of fluorescence compared with that in the NP and C (Table 2). Yield was significantly affected by shading and showed significant effects of P addition and the interaction between N and P addition (df = 1, *F* = 12.4, *p* = 0.001). Average leaf area per shoot was significantly affected by the addition of nutrients when coupled with a decrease in light and by the interaction of temperature and nutrient addition (Table 3).

Essential oil quantity and composition

The total quantity of essential oil components differed significantly across treatments (Fig. 2), with the quantity of essential oil lowest in the P and SC treatments (>80% lower than control) and highest in both the C and CNP. The quantity of essential oil did not significantly correlate with leaf nutrients or the physiological parameters measured across treatments (data not shown). Two-way ANOVAs of the quantity of essential oil in response the specific treatments and their interactions (Table 4) showed significant effects of reduced light (df = 1, *F* = 5.484, *p* = 0.023) but not increased temperature (df = 1, *F* = 1.695, *p* = 0.198), despite nearly 40% reductions in the two warming treatments. The addition of nitrogen and phosphorus alone were not significant, but the interaction was (Table 4, df = 1, *F* = 4.21, *p* = 0.044).

An average of 17 components was detectable at greater than trace levels in the headspace of *R. tomentosum* ssp. *subarcticum* for all treatments (Table 5, Supplementary Figure S2[2]). The SNP had the lowest number of compounds (12) and NP had the highest number of compounds (22). The number of compounds was positively correlated with the foliar percent phosphorus (Pearson's Correlation *r* = 0.207, *p* = 0.013) and negatively correlated with the C:N ratio (*r* = –.263, *p* = 0.002). The number of compounds was correlated negatively

Fig. 2. Total quantity of essential oil detectable in the headspace of samples of *Rhododendron tomentosum* ssp. *subarcticum* by treatment: C, Control; N, Nitrogen; P, Phosphorus; NP, Nitrogen + Phosphorus; GC, Warming; GNP, Warming + Nitrogen + Phosphorus; SC, Shade; SNP, Shade + Nitrogen + Phosphorus. Units: Arbitrary. Bars represent means ± SE.

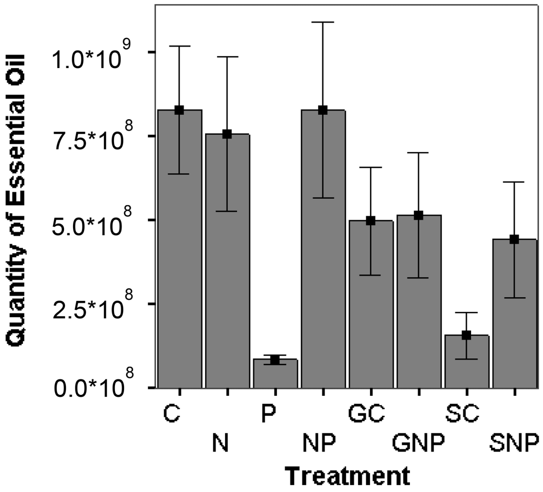


Table 4. Two-way analysis of variance (ANOVA) results for the effect of light attenuation, nutrient addition, the interaction of light attenuation and nutrient additions, increased temperature and nutrient addition, and the interaction of increased temperature and nutrient additions, and nitrogen addition, phosphorus addition, and the interaction of nitrogen and phosphorus additions on the quantity of essential oil and the number of compounds in the essential oil of *Rhododendron tomentosum* ssp. *subarcticum*. *p* values < 0.05 are highlighted in bold.

		Essential oil quantity		Number of compounds	
Effect	df	*F*	*p*	*F*	*p*
Light	1	5.48	**0.023**	7.36	**0.009**
Nutrient	1	0.394	0.532	0.208	0.650
Light*Nutrient	1	0.408	0.525	0.375	0.543
Temperature	1	2.50	0.118	0.723	0.398
Nutrient	1	0.001	0.971	2.86	0.095
Temperature*Nutrient	1	0.002	0.962	0.311	0.579
Nitrogen	1	2.83	0.097	1.98	0.164
Phosphorus	1	2.87	0.094	0.136	0.714
Nitrogen*Phosphorus	1	4.21	**0.044**	0.619	0.434

with A_{max} ($r = -0.270$, $p = 0.002$) and weakly negatively correlated with F_v/F_m ($r = -0.157$, $p = 0.06$). The quantity of essential oil and the number of compounds in the essential oil were not correlated. The number of compounds decreased with reduced light (df = 1, $F = 7.361$, $p = 0.009$), and unlike the quantity of essential oil, did not seem to be as negatively affected by the addition of phosphorus alone (Table 4).

When the components were examined independently, several compounds were found to be driving the variation in the quantity of essential oil. The effect of the treatments on the essential oil content was partly explained through partial correlations, with camphene

Table 5. Average percent of components of the essential oil of *Rhododendron tomentosum* ssp. *subarcticum* based on treatments (Rt, Retension time in the gas chromatograph; C, Control; GC, Warming; GNP, Warming + Nitrogen + Phosphorus; N, Nitrogen; P, Phosphorus; NP, Nitrogen + Phosphorus; SC, Shade; SNP, Shade + Nitrogen + Phosphorus). Values are mean ± SE.

Rt	Compound	C	GC	GNP	N	P	NP	SC	SNP
Number of Compounds		18 ± 3	14 ± 2	21 ± 3	20 ± 3	15 ± 3	21 ± 3	12 ± 1	12 ± 2
4	α-pinene	6.36 ± 1.24	3.24 ± 0.55	3.91 ± 0.50	5.12 ± 0.80	2.77 ± 0.66	7.29 ± 0.83	8.62 ± 1.58	2.31 ± 0.80
4.2	camphene	8.31 ± 2.21	1.38 ± 0.41	1.30 ± 0.33	6.36 ± 1.51	2.94 ± 1.16	8.10 ± 1.75	9.09 ± 2.94	0.40 ± 0.27
4.6	unknown 1	0.322 ± 0.23	0.792 ± 0.27	1.40 ± 0.52	0.467 ± 0.20	0.708 ± 0.35	0.763 ± 0.28	0.145 ± 0.15	3.99 ± 1.13
4.7	β-pinene	6.72 ± 1.1	3.55 ± 0.94	5.70 ± 0.85	5.64 ± 0.90	3.95 ± 0.91	6.86 ± 0.51	6.39 ± 0.98	0 ± 0
5.2	α-phellandrene	0.288 ± 0.18	0.401 ± 0.28	0.0275 ± 0.02	0.123 ± 0.04	0.045 ± 0.02	0.153 ± 0.09	0.036 ± 0.036	0 ± 0
5.4	allocymene	5.84 ± 1.25	2.09 ± 0.99	1.72 ± 0.66	3.48 ± 0.80	6.03 ± 3.30	9.73 ± 1.60	13.42 ± 5.30	9.6 ± 3.50
5.6	cymene	47.8 ± 6.10	55.9 ± 2.71	51.6 ± 4.23	42.0 ± 4.90	51.3 ± 5.30	35.7 ± 2.99	39.8 ± 4.32	56.3 ± 3.58
5.7	unknown 2	2.27 ± 0.82	1.007 ± 0.57	2.34 ± 0.81	3.23 ± 0.85	0 ± 0	0.572 ± 0.41	0 ± 0	0 ± 0
5.8	unknown 8	11.2 ± 2.68	20.6 ± 4.06	12.7 ± 2.65	13.7 ± 4.01	16.5 ± 3.94	9.65 ± 2.21	11.7 ± 3.24	17.3 ± 3.23
6.3	sabinene	1.86 ± 0.43	1.35 ± 0.42	2.17 ± 0.43	2.17 ± 0.51	1.33 ± 0.54	2.53 ± 0.41	1.49 ± 0.48	1.16 ± 0.420
9.3	unknown 3	0.110 ± 0.08	0.220 ± 0.20	0.025 ± 0.02	0.054 ± 0.04	0.271 ± 0.27	0.199 ± 0.209	0 ± 0	0 ± 0
10.1	terpinen-4-ol	2.36 ± 1.46	1.363 ± 0.96	4.52 ± 1.95	2.78 ± 0.75	1.72 ± 0.82	4.09 ± 1.51	1.49 ± 0.81	0 ± 0
10.5	unknown 5	0.463	0.725 ± 0.25	1.32 ± 0.33	1.17 ± 0.25	0.483 ± 0.18	0.719 ± 0.24	0.632 ± 0.28	0 ± 0
11.1	unknown 6	2.88 ± 0.80	0.352 ± 0.16	0.561 ± 0.18	3.07 ± 0.97	1.47 ± 0.59	4.02 ± 1.11	1.41 ± 0.41	1.17 ± 0.410
11.7	terpinene	3.22 ± 1.07	8.17 ± 1.89	11.8 ± 1.90	11.4 ± 1.85	10.0 ± 3.17	7.03 ± 1.49	5.57 ± 1.99	3.30 ± 1.53
	n	20	20	20	20	20	20	13	10

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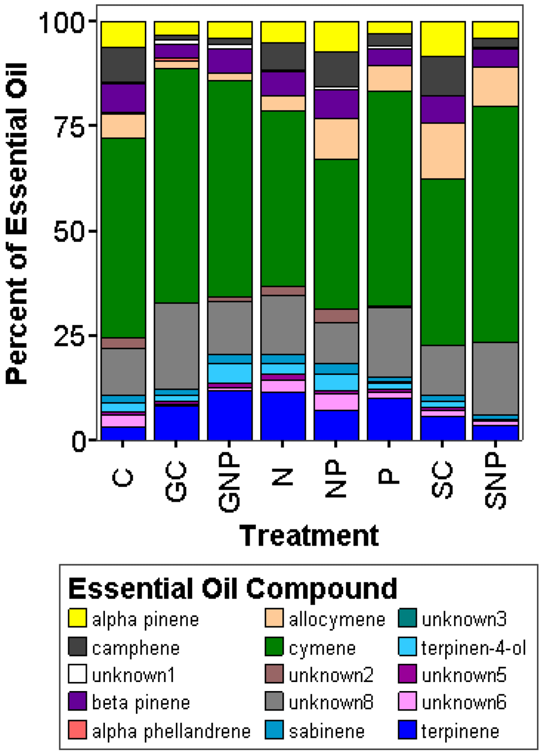
Fig. 3. Representation of the percent of each compound in the essential oil of *Rhododendron tomentosum* ssp. *subarcticum* by treatment (C, Control; GC, Warming; GNP, Warming + Nitrogen + Phosphorus, N, Nitrogen addition; NP, Nitrogen and Phosphorus addition; P, Phosphorus addition; SC, Shade; and SNP, Shade + Nitrogen + Phosphorus). Segment size within stacked columns for each treatment represents mean percentage of the replicate blocks for each color-coded compound.



($R^2 = 0.208$, allocymene ($R^2 = 0.188$), and unknown compound 6 ($R^2 = 0.138$) having the largest influence on treatment difference (Fig. 3). These three compounds were at very low levels in the warming treatments (GC and GNP) compared with their much greater levels in the other treatments (Fig. 3). Unknown monoterpene 2 also explained over 18% of the variance, with the highest values found in the three treatments with highest total essential oil: C, N, and CNP. Unknown monoterpene 3, α- phellandrene, cymene, unknown 8, sabinene, and terpinenol did not significantly affect the relationship of the treatments to the essential oil.

Discussion

Total essential oil content

We found very large differences in essential oil contents across treatments, with >80% reductions relative to the controls in two treatments and >35% reductions in three others. Despite these reductions, the hypothesis that enhanced nutrient levels and decreased carbon gain would result in decreased essential oil concentrations in *R. tomentosum* ssp. *subarcticum* was only partially supported. The two strongest decreases were driven by different factors: the effects of light for SC and the interaction between N and P treatment for P addition (Table 4).

Reduced growth demand in low light often leads to increased leaf nutrient concentrations (Dormann 2003), and leaf N and P values of the shade control were slightly greater than those of the overall control, so the decrease in shade cannot be attributed to increased nutrient content. Consistent with response to a low light environment, the SC treatment had significantly reduced Yield and the lowest A_{max} of all the treatments, but relatively high values for F_v/F_m, likely because the shade treatment protected against photoinhibition (Oberbauer and Starr 2002). Total leaf area of shoots was not significantly affected by shade but was significantly related to nutrient content in the light by nutrient ANOVA. Typically, mean leaf size in plants increases in shade to maximize light capture (Jackson 1967). In the case of *R. tomentosum*, sun leaves are normally rolled and folded under the edges, whereas shade-grown and luxury-growth leaves are fully expanded without rolling and with much greater area than sun leaves. That leaf size in the SC treatment did not increase along with the reduced A_{max} suggests that carbon resources were limited in the SC treatment. The SNP treatment plants also had reduced essential oil content compared with overall controls, but significantly higher values than the shade controls, contrary to our hypothesis. Shading and nutrient addition were expected to decrease carbon-based defensive compounds by increasing N content (Dormann 2003). The SNP treatment plants had higher nutrient content, photosynthesis, fluorescence, and leaf area than the shade controls, and along with the finding that nutrient contents of shade leaves were similar to those of the controls suggests that the SC treatment was both carbon and nutrient limited. Enhanced nutrient availability in light-limited environments has previously been shown to improve shade tolerance (Grubb et al. 1996). In both the SC and SNP of the present experiment, *Rubus chamaemorus* became the dominant species in these plots, consistent with the results reported for the prototype experiment by Chapin et al. (1995). In some of the shade treatment plots of our study, *R. tomentosum* ssp. *subarcticum* had completely died out.

The very low essential oil content in the P treatment was accompanied by C:N ratios and leaf area similar to the overall controls, low A_{max}, the lowest fluorescence parameters of all treatments, and strongly elevated P content. Changes in N:P ratios are often observed to affect production of secondary metabolites, especially flavonoids, which accumulate at low P levels (Yang et al 2018). The C:N:P balance presents an important regulating factor in the general physiological status of plants (Loomis 1932). The addition of P alone apparently disturbed metabolic balance in *Rhododendron* in this N-limited ecosystem, strongly affecting photosystem II processes. Results from the study by McLaren and Buckeridge (2019) found clear effects of P alone and NP interactions on species cover in the same plots used in the present study after 26 years. These results contrast strongly with a fertilization study of plant defensive compounds on subarctic heath that confirmed a prediction that P addition would show minimal effects across three evergreen, three deciduous, and two grass species (De Long et al. 2016).

Contrary to our hypothesis, N + P addition showed no effect on essential oil content in the warming treatments, with a consistent but not significant approximate 40% reduction in essential oil content in warmed alone and warmed plus N + P compared with that of controls. Warming decreased leaf nutrients leading to values for the GC treatment to be the lowest of all the treatments and those of the GNP treatment to be similar to the overall controls. Tundra warming experiments studies have repeatedly shown increased leaf size in response to increased air temperature (e.g., Arft et al. 1999). Dilution of nutrients in larger leaves is consistent with growth stimulation by aboveground warming. Furthermore, in the prototype study by Chapin et al. (1995), increased temperature decreased nutrient availability at the plot level by nutrient sequestration in fast growing plants. This latter finding is consistent with the concept that the stimulation of community growth triggered by warming and the applied nutrients resulted in balanced nutrient uptake

(Shaver et al. 2001). The two warming treatments had the highest photosynthetic rates and fluorescence parameters on the high end of all treatments, likely as a result of warming stimulation (Chapin and Shaver 1996; Nybakken et al. 2008). With decreased nutrient availability and increased photosynthesis, content of carbon-based defensive compounds might be expected to increase. That the warming plus nutrient addition resulted in the same levels of essential oil content as the GC treatment was unexpected. However, the absence of some compounds in the warming treatments raises the possibility that the increased temperature volatilized some of the measured compounds or their precursors (Tatro 1973). Although the average temperature increase resulting from the warming vs. controls is relatively small (approximately 3.5 °C), on sunny days near solar noon, temperatures in greenhouses at the site can be as high as 10 °C greater than that in controls (Chapin et al. 1995). Temperatures near these levels have occurred under ambient conditions during recent episodic heat waves (Toolik Field Station EDC 2016), so the potential of high temperatures reducing essential oil content in the future is a concern. By contrast, production of many secondary compounds is stimulated by higher temperatures (Yang et al. 2018). The production of isoprene, the base component of terpenoids, increases with temperature and is hypothesized to serve in thermal protection of leaves (Sharkey et al. 2008). Whether the lower quantities of terpenes in the warmed treatments is a balance of volatilization with temperature-mediated metabolic effects on terpene production is unknown, but the result is a reduction in compounds that might serve as defense or medicinal/food resources. Previous studies of warming effects on production of defensive compounds in high latitude or alpine plants have been mixed; reductions were found for boreal tree seedlings (Kuokkanen et al. 2001) and some Arctic plant species (Hansen et al. 2006), but no response was seen for the High Arctic shrub *Salix polaris* (Dormann 2003) and four of five vascular plant species in the subarctic alpine (Nybakken et al. 2011). A gradient study in alpine Norway found that carbon-based secondary compounds decreased with increasing elevation (Asplund et al. 2021). However, the defensive compounds in these studies did not include volatile compounds such as terpenes.

Addition of N and N + P did not strongly affect the amount of essential oil relative to the overall controls, contradicting our hypothesis. *Rhododendron* is reported to be among the least responsive species in the dominant tussock tundra plant community in terms of nutrient accumulation with fertilization (Chapin et al. 1995). Nevertheless, in both treatments, C:N ratios were strongly reduced whereas P levels increased. Photosynthesis and fluorescence levels were similar to those of the controls. Chapin and Shaver (1996) found the highest A_{max} for *Rhododendron* after 9 years in their experiment with NP addition, and similar to our measurements found relatively low photosynthetic rates overall and small absolute but large relative differences in response to treatments. Overall, the patterns for N content in our study were essentially the same as those found for the comparable treatments reported by Chapin and Shaver (1996). Increased levels of nitrogen are predicted to decrease carbon-based defensive compounds (De Long et al. 2016), although Hansen et al. (2006) found opposite responses for phenolics in some species in response to a long-term experiment similar to the present study. In the High Arctic shrub *Salix polaris*, phenolics decreased with increased leaf N caused by shading from expanded growth, but fertilization with N alone had no effect (Dormann 2003).

Composition of essential oil

No correlation was found between the number of compounds and the production of total essential oils, although the shade and phosphorus addition treatments that had the lowest total quantity were on the low end of the range of total number of compounds. In a latitudinal transect study of the same species, the number of compounds was positively correlated with total quantity of compounds (Baldwin 2003). The negative relationships found between F_v/F_m and A_{max} and the number of compounds suggests that in general, plants with lower stress levels as indicted by stronger photosynthesis indicators tend to produce a lower diversity of compounds. The number and quantity of compounds were also strongly reduced in the shade treatments, potentially driving the aforementioned relationship. An important finding of our research was that the individual components of essential oil did not all respond to a treatment in the same direction; some compounds increased whereas others decreased. This finding challenges the general concept that changes in the content of carbon-based defensive compounds in response to resource changes would be congruent. This finding was not restricted to the warming treatments, in which some compound concentrations might have been influenced by volatilization at high temperatures. It is possible that a fixed amount of resources is differentially allocated among the various terpenes, an idea that is consistent with the absence of a correlation between total amount of essential oil and number of compounds. Similar differential responses with treatment have been shown to be the case across classes in a long-term study in Arctic Sweden (Hansen et al. 2006). In that case, the production of individual compounds may be responding to the direct effects of N and P concentrations on specific synthesis pathways (De Long et al. 2016). However, our study was limited to a single compound class, terpenoids, that previously have been reported to be generally unresponsive to changes in carbon availability (Koricheva et al 1998).

Ecosystem changes in response to treatment

The results of our experiment clearly indicate that changes in resources will have large effects on the expression of essential oil by *Rhododendron tomentosum*. However, as a result of the large changes in ecosystem structure and function that occurred in response to the 14 years of treatments, ascribing the changes in essential oil quantity and composition to specific factors is difficult. Increased levels of resources generally result in increased productivity (Crane and Banks 1992). However, sufficient time has passed that the resource manipulations have altered the community composition to such an extent that resources available to *Rhododendron*, including unmanipulated resources, likely decreased in some treatments relative to the control because of factors such as decreased light levels and nutrient availability of unamended nutrients and increases in denitrification and water stress as indirect effects of the treatments (Chapin and Shaver 1996).

Moist tundra ecosystems are typically nutrient limited because of low decomposition in cold, wet soils with recalcitrant litter (Billings 1987). According to Chapin et al. (1995), the nitrogen limitation to plant production at the study site is stronger than that of low light or low air temperature. The prototype experiment, described in the “Methods and Materials” section that started 8 years earlier than the current study, showed large community changes in response to the treatments after 9 years (Chapin et al. 1995; Chapin and Shaver 1996). The NP and NP warming treatments showed loss of lichens and bryophytes and large increases in biomass and the canopy cover of the deciduous shrub, *Betula nana*. Warming alone also showed loss of mosses and lichens and increases in both evergreen and deciduous shrubs, whereas shade alone lost approximately 40% of the community biomass with the strongest decrease in deciduous and evergreen shrubs. Similarly, in the current study, the N and NP plots had a dense overstory of deciduous shrubs after 14 years of treatments (T.A. Baldwin, personal observation, 2002). After nine years of treatments in the prototype experiment, *Rhododendron* peak-season biomass decreased slightly in shaded plots (−20%), decreased strongly with added N+P (−36%) and with warming + N + P (−75%), and increased in warming only plots (+46%). Some *Rhododendron* cover data have been published from the LTER experiment; cover was not different from controls after

10 and 20 years in the warming (GC) treatment (Sistla et al 2013), and after 26 years cover of *Rhododendron* was similar among the C, N and P treatments, but reduced to near zero in the NP treatment (McLaren and Buckeridge 2019). The decrease in the growth of *Rhododendron* in the present study for the GNP treatment is consistent with the much-reduced leaf area in GNP compared with the GC, as a result of competition and shading from faster growing shrubs. One of the noteworthy changes in response to these treatments detected by Chapin and Shaver (1996) was decreased shoot size and increases in shoot numbers during the experiment in the dwarf evergreens, raising the possibility that total essential oil production could have remained the same despite reductions in shoot leaf area and photosynthesis. In an analysis of the fertilization effects on the prototype experiment after 15 years, the change in species composition in response to fertilization distinctly impacted nutrient cycling in addition to the impact of the annual fertilizer additions (Shaver et al. 2001). That change in nutrient cycling was largely the consequence of *Betula nana* sequestering the majority of the system nitrogen in leaves and wood as it grew taller to dominate the canopy. In our study, there was an observable increase in the canopy height of *Rhododendron* in the treatments with added nitrogen as it became shaded within the middle of the greatly expanded canopy of the deciduous shrubs. Similar to the present study (T.A. Baldwin, personal observation, 2002), the community changes within the shaded + fertilizer treatment of the prototype study were to the point of near complete exclusion of all species except *Rubus chamaemorus*). After 25 years of treatment, *Rubus* cover has also expanded greatly in the NP treatments whereas *Betula* cover has decreased (McLaren and Buckeridge 2019). These changes likely reduced the detectability of the direct effects of the treatments. Only shade and the N*P interaction triggered by the P addition significantly affected quantity of essential oil, and shade affected the number of compounds. The strong correlation between number of compounds and leaf C:N ratio in the absence of any significant effects of N in the two-way ANOVAs supports the view that the direct nitrogen response is obscured by indirect consequences of ecosystem changes.

Similar to the present study, most studies of defensive compounds in response to experimental manipulation in Arctic and alpine plants have been snapshots from existing field experiments after several years of treatment that include community and structural changes. In that sense these experiments are generally comparable with the current study and provide a contrast to experiments on monocultures or in containers or field experiments in their first or second year. These ecosystem-level experiments provide more realistic projections of what the results of changes in resources would actually mean for defensive compounds of the study species. However, because of disconnects and lag between aboveground and belowground processes, prediction of what the future plant communities will look like is difficult. In the case of the present study, which is still ongoing, community composition is still changing after 30 years.

Conclusions

The work presented here evaluated the effects of altered resources on the production and composition of terpenes, carbon-based defensive compounds in the essential oil of an important Arctic and boreal species, *Rhododendron tomentosum* ssp. *subarcticum*. Our results suggest that under field conditions, changes in temperature, nutrient availability, and light will result in changes in essential oil production. The composition and quantity of essential oils were a complex combination of responses, and it was difficult to identify all the drivers within this experimental framework. Unexpectedly, phosphorus addition by itself in this nitrogen-limited system also strongly reduced the quantity and diversity of compounds. Phosphorus additions at the levels of this experiment are not going to occur in response to climate warming, but as thaw depths increase with warming, exposure of mineral soil will provide a new source of phosphorus (McLaren and Buckeridge 2019). Shading also had large effects, strongly decreasing the quantity and number of compounds produced, likely through decreased carbon gain. Increased shade is the most likely scenario for *Rhododendron* in the future as the climate warms and shrubs increase canopy cover. Low C:N ratios (high N) were associated with a high diversity of compounds. After 14 years of ecosystem response to the treatments, the observed changes are likely driven in part by directional ecosystem changes that have taken place in response to the original treatments as well as direct response to the original manipulations, such as shading by heavy shrub cover. Climate changes will be accompanied by some of these ecosystem changes, and our work has shown that essential oil production and diversity will respond as well. Our study is one of the first investigations of the production of defensive compounds by tundra species that specifically focused on terpenes, which were generally thought to be unresponsive to resource changes, but clearly warrant further research.

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