

2014 Project Abstract

For the Period Ending June 30, 2017

PROJECT TITLE: Impacts of forest quality on declining Minnesota moose.

Project Manager: James D Forester

Organization: University of Minnesota

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 05I and Date of Work Plan Approval: June 4, 2014

APPROPRIATION AMOUNT: \$300,000

AMOUNT SPENT: \$277,414

AMOUNT REMAINING: \$22,586

Overall Project Outcome and Results

We examined characteristics of land cover and forage quality that could be affecting the declining Minnesota moose population at multiple spatial and temporal scales. At a broad spatial scale, we found that the landscape of NE Minnesota has changed over 18 years, both in the composition (e.g., more coniferous and less mixed-wood forest) and arrangement (e.g., decreased fragmentation of coniferous forest and increased fragmentation of mixed-wood forest) of forested land-cover types. At the scale of the moose survey unit (2.8 x 5 miles), some of these changes appear to be related to moose population dynamics. Specifically, moose tended to have higher population growth rates in cooler areas and in survey units that had more young and mixed-wood forest, less coniferous and deciduous forests, and less fragmented forested wetlands. We found that, during summer, moose are in fact altering their behavior to seek out mixed-wood forest at the hottest times of the day, and because we found that forage availability differs both by cover type and by location in moose range, these decisions may be affecting diet. Further, because the diets of animals that died were different from those of live animals, we suspect that the availability of high-preference foods may be critical. Because several independent sources of data all point in a similar direction, we recommend a large-scale, long-term experiment to explicitly test how different combinations of land cover and food availability may be affecting moose habitat use and population dynamics. Specifically, we suggest working with forest managers to harvest blocks of forest stands to manipulate overstory and understory features that appear to be important to moose. Monitoring the success of these manipulations with collared animals and camera traps over a 5-10 year period could help determine how to best manage forest landscapes for a healthy moose population.

Project Results Use and Dissemination

This research has been presented 15 times at national or international research conferences or invited seminar series. The research team has worked with the Bell Museum to contribute information related to the moose diorama and also provided an extensive interview to the "Access Minnesota" radio show. Three scientific articles have been published so far, and the research team is working with MNDNR and tribal biologists to discuss the results and implications of this work. Finally, 12 undergraduate students, five graduate students, and three postdoctoral researchers received training as part of this project;

results from this research have been added into teaching materials in two required Fisheries, Wildlife, and Conservation Biology courses at UMN.



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2014 Work Plan Final Report

Date of Report: 31 August 2018
Date of Next Status Update Report: Final Report
Date of Work Plan Approval: 4 June 2014
Project Completion Date: 30 June 2017

PROJECT TITLE: Impacts of forest quality on declining Minnesota moose.

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Location: St. Louis, Lake, and Cook Counties (see Figure 1).

Total ENRTF Project Budget:	ENRTF Appropriation:	\$300,000
	Amount Spent:	\$277,414
	Balance:	\$22,586

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 05I and Date of Work Plan Approval: June 4, 2014

Appropriation Language:

\$300,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota in cooperation with the Department of Natural Resources to link regional patterns of moose abundance through time to the distribution of food and cover and determine if this distribution affects the diet and survival of individual moose. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Impacts of forest quality on declining Minnesota moose.

II. PROJECT STATEMENT:

The Minnesota moose population is declining dramatically and has become a growing concern for conservation. In addition to being an iconic species of northern Minnesota, moose are keystone herbivores that are an important component of Minnesota's forested ecosystems. The specific mechanism causing their rapid decline has not been fully uncovered because many factors affect how well moose survive and reproduce. Ultimately, the most important tool available to natural resource managers is their ability to manipulate the spatial distribution and diversity of high-quality habitats (Figure 2). Management decisions will clearly benefit from scientific guidance to ensure manipulations have maximum impact on stabilizing the moose population in Minnesota.

The Minnesota Department of Natural Resources (MNDNR), the Grand Portage Band of Lake Superior Chippewa (GPBLS), and the University of Minnesota began a moose tracking effort in 2013 to determine cause-specific mortality within the moose population (128 GPS collars were deployed). In addition, Dr. Ron Moen (NRRI) is working on a moose habitat restoration project in which he is assessing how food availability, quality, and consumption by moose changes in forests with different disturbance histories. We propose to build upon both of these LCCMR-funded research projects to explore how the landscape context in which individual animals live can directly affect the animals' diet and their subsequent body condition and mortality risk. Understanding how forest age, structure, and composition can affect the distribution of food and cover (and thus impact the movement patterns of moose) is critical to inform broad-scale management efforts that are aimed to improve the forest landscape for moose and thus stabilize the population.

Our *broad aim* is to link the behavior, diet, and survival of moose to the spatial distribution of food and cover. Our team will build upon existing moose research in the state to address two primary *research goals*:

- 1) *Regional Scale*: Link regional patterns of moose abundance through time to the geographic distribution and relative forage quality of different land-cover types and forest stand ages.
- 2) *Local Scale*: Determine if the distribution of resources affects the diet of individual moose and whether dietary differences among animals are associated with variation in body condition or mortality risk.

This will be the first study to link the movement behavior and landscape context of individual moose (e.g., the distribution of food and cover within an animal's home range) to the animals' diet, body condition, and mortality risk. It will allow us to place the moose movement, mortality, and forage quality data already being generated by LCCMR funding into a detailed ecological and behavioral framework that will provide critical and timely insight into the causes of the moose population decline.

III. PROJECT STATUS UPDATES:

Project Status as of 1 December 2014:

We had a successful first field season – collecting several thousand plant samples at 140 sites distributed across moose range in northeastern Minnesota. We have also begun running stable isotopic analysis of hair previously collected from moose and the initial results confirm the large amounts of variability we saw in our pilot analysis conducted last year. Our graduate student has found other funds to support his stipend, so we are using the remaining funds initially set aside for his summer salary to support a postdoctoral researcher for 6 months next year. This person has already been working on moose in Ontario and will be able to do the critical initial organization of the moose movement data and begin to develop statistical models that link resource availability to diet composition.

Our work with the FIA data is also proceeding as planned. We have summarized annual estimates of land-cover compositions within each moose survey unit and have developed initial population models to understand if changing land cover is linked to dynamics in the moose population. We are now working with remote sensing labs to begin the process of developing a time series of Landsat images within our study area.

LCCMR approved the amendment to add the postdoctoral scholar to the personnel budget category -December 17, 2014.

Amendment Request 30 April 2015:

We would like to shift the GIS and Statistical consulting funding in Activity 1 “Professional/Technical Service contracts” to “Personnel” in Activity 1. We were originally planning on getting this done through an external contract but we decided that using UMN facilities would produce a more consistent product since they specialize in MN satellite analysis. We are requesting that we use \$25,000 to pay 3.5 months of GIS analyst time (split between the Knight and Falkowski labs) to produce the satellite products we need for the next phase of this project. We have made no changes in the work plan as this is simply a shift in the budget that will yield the same product. *Approved by the LCCMR 5/1/2015*

Project Status as of 31 May 2015:

We hired a postdoc for this project to compile the moose movement data collected by many researchers in the state. He has finished cleaning these data and is well into his analysis of how moose change their selection of landscapes based on time of day and changes in daily temperature. We are preparing for another field season to collect additional vegetation data and control points to help with validating the land-cover classification we have commissioned from the Knight lab. Our initial results from the stable isotope analysis show that we can detect differences in isotopic ratios in different cover types and across a summer temperature gradient in the study area; we see similar patterns in the moose hair that we have analyzed. As we continue to collect more data, we will use this information to build models to estimate the diet of individual moose and relate this to body condition, behavior, and survival.

Project Status as of 31 January 2016:

Our second field season went very well. We focused on collecting aquatic vegetation during June and continued to collect data on forage abundance and composition throughout the study area for the rest of the summer. Although our progress has been slightly hampered by a malfunctioning mass spectrometer and having one of our remote-sensing colleagues move to another university, we are still making progress with the analysis. Our first manuscript was accepted for publication in *Landscape Ecology*, and in it we show that Minnesota moose strongly alter their selection of land-cover composition based on ambient temperature; this effect was not as strong in moose followed in Ontario where forest composition is more mixed (i.e., foraging opportunities are closer to thermal cover). We will use the results from this paper to help drive our analysis of landscape patterns at the individual and population levels.

Project Status as of 31 May 2016:

We conducted a winter field season to collect browse samples at our field sites. In the lab, we have been focusing on finishing our stable isotope analyses and our initial data set has shown that the moose in the state eat markedly different diets depending on where they live; these differences are even more dramatic when seasonal changes in diet are examined. Now that we have our improved land-cover classification, we have begun examining how forage composition and availability differs among cover types and by disturbance history. We are currently preparing for our final field season in which we will primarily collect plant samples and forage biomass data from forested wetlands.

Project Status as of 1 May 2017:

We received the historical land-cover data for moose range and are in the process of analyzing how the composition and arrangement of different land-cover classes change across moose survey units. Half of our stable isotope and metabolomics samples are still being analyzed by the labs; however, we expect the data to arrive in the next two weeks. Our spatially-explicit population estimation model has shown great improvement over previous versions and we continue to refine it using the newly received historical land-cover data. Our initial model to predict forage biomass across moose range using LiDAR has limited predictive power due to high variation in the observed field data; we are currently testing whether adding in additional data layers (land cover, topographic position, soils) will improve the model. Using data collected from recovered moose collars, we have developed an approach to estimate the amount of time an animal spends foraging at different times of the day; our initial results indicate that, during summer, moose actively forage the most during dawn and dusk. This suggests that the cover types preferred by the animals at these times will be the most important contributors to the overall summer diet of the moose. Because our data have been delayed due to processing issues in the labs we contracted with, we are slightly behind our initial schedule. We anticipate being able to complete the work on time if we are able to move some of the unspent budget to personnel.

Amendment Request 1 May 2017:

Personnel (Wages and Benefits): \$47,815

Because so much of the contracted data (historical land-cover layers and plant chemical composition results) have been late to arrive, we need extra help on the analysis end. We request the unspent and unencumbered amounts in the other budget sections (\$47,815) to be transferred to Personnel to fund additional work on the final analysis (two full time and one part-time graduate student, a postdoc, and one month of PI Forester's time). One graduate student worked for one month to develop models that allow us to identify moose behaviors in different areas of the landscape (i.e., proportion of time foraging in wetlands), another student is currently working full time on refining the moose diet composition models, and a third student is developing a program that will help us predict how moose distributions will change in response to different distributions of land-cover and other resources. The postdoc is refining forage availability maps for moose range and also developing the spatially-explicit population estimation model; Forester will continue to work on a population dynamics model that will further refine the spatially-explicit estimates. For the graduate students and postdoc, we are making this request retroactively because although we had discussed these changes with LCCMR staff and prepared our amendment request earlier in the year, the report was mistakenly not sent out before leaving for the field this winter.

Professional/Technical/Service Contracts: -\$2,084

In 2013, we began running stable isotope analysis on plant and animal tissue at the stable isotope lab in the Department of Earth Sciences at the University of Minnesota; however, multiple stoppages due to a wide range of technical issues slowed progress for extended periods of time. In mid 2014, a new isotope lab in the Department of Soil Sciences was up and running, and to help minimize our dependence on the lab in Earth Sciences, we decided to run samples in this lab as well. Prior to sending new samples to this lab we decided to run a series of replicates to ensure that we would not experience any lab-specific bias. Unfortunately, this lab is tuned to running soil samples and the nitrogen values we received were well outside normal $\delta^{15}\text{N}$ values of plant and animal tissues. In mid 2015, the stable isotope laboratory in the Department of Earth Sciences at the University of Minnesota began to occasionally encounter severe column issues that resulted in unreliable nitrogen values, which is a critical part of our analysis, and by mid 2016, this lab decided to stop running samples until the issue had been completely resolved. At this point we were beginning to get too far behind schedule and began to look for other stable isotope labs to analyze our samples in case the UMN machine took too long to repair. Unfortunately, we could not find other labs in the state of Minnesota that run outside samples. After comparing prices at three different isotope laboratories (the University of California at Santa Cruz, University of Utah, and University of California at Davis), we found the lowest price at the University of California at Santa Cruz (\$11.74/sample compared to \$13.13/sample and \$12.50/sample at the other labs — note that this price includes weighing out of the samples, a service not included in the

\$9/sample charged at UMN, so this price is not substantially higher than our original in-house rate). The Santa Cruz lab is a national leader in stable isotope ecology and specializes in the analysis of a broad range of materials, including plant and animal tissues. In addition, they have an excellent reputation for working with large numbers of samples and with individuals from outside the University of California system. We contacted the University of California at Santa Cruz in July of 2016, and sent them approximately 50 replicate samples. Within two weeks we received these replicate data and they aligned extremely well with the same samples run in the Department of Earth Sciences at the University of Minnesota. We continued to wait for the UMN stable isotope lab to come back online; however, by the end of 2016 the machine was still down so we decided to send our remaining samples to Santa Cruz. We are asking for a retroactive approval for this switch because Forester did not realize that approval was needed before changing service providers. The Santa Cruz Stable Isotope Lab is currently processing our samples (2191 samples for \$25,719) and should have the results to us in the next few weeks. To get more information on chemical composition of forage, we processed samples using liquid chromatography–high resolution mass spectrometry (LC–HRMS) in the UMN Metabolomics lab (\$15,000). Finally, the Knight Lab in UMN preferred to charge us under a Professional Contract instead of us paying salary as originally planned (\$5,000). We request the balance of this portion of the budget (\$2,084) to be transferred to Personnel.

Equipment/Tools/Supplies: -\$385

Because much of our sampling was moved to an external lab, we did not need to purchase as much lab equipment and supplies as expected. Of the difference, \$5845 is being reallocated to pay for the non-capital GPS receiver; we request the balance (\$385) to be transferred to Personnel.

Capital Expenditures: -\$5,845

Because the GPS system included two different parts (a data recording tablet and a high-precision GPS receiver), they could not be listed as capital expenditures so \$5845 was paid out of the Equipment budget. We request the budget for Capital Expenditures (\$5,845) be transferred to Personnel.

Travel expenses in Minnesota: -\$39,500

We were able to find much cheaper than expected accommodation for our field crew, and ended up requiring the vehicle for less time during the year. We also needed to hire the crew at a higher hourly rate to be competitive so we did not pay for meals over the entire period. As a result of these reductions, this budget line was not heavily used. We request the balance (\$39,500) be transferred to Personnel.

Project Status as of 31 June 2017:

We are still waiting on the results from Santa Cruz. The UMN stable isotope lab realized they had not processed some of our samples they had on file, so they have sent them to Santa Cruz for processing. As a consequence, we moved \$2,178 to that contract.

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animals, we suspect that the availability of high-preference foods may be critical. Because several independent sources of data all point in a similar direction, we recommend a large-scale, long-term experiment to explicitly test how different combinations of land cover and food availability may be affecting moose habitat use and population dynamics. Specifically, we suggest working with forest managers to harvest blocks of forest stands to manipulate overstory and understory features that appear to be important to moose. Monitoring the success of these manipulations with collared animals and camera traps over a 5-10 year period could help determine how to best manage forest landscapes for a healthy moose population.

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IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Linking moose abundance to broad-scale distributions of food and cover that change across space and through time.

Description: We hypothesize that broad-scale changes in the arrangement (rather than simply the abundance) of important cover types (e.g., young and mature forest, wetlands) measured at the level of four townships or larger will be linked to changes in moose abundance. Areas dominated by one cover type (e.g., young forest) will be avoided in preference for areas that contain a mixture of cover types that provide reduced distances between thermal cover and high quality forage. We will use a combination of USFS Forest Inventory and Analysis (FIA) data and satellite data (both collected repeatedly over the last 13 years) in conjunction with data from the MNDNR moose survey to examine how the moose population has responded to changes in distributions of resources across its Minnesota range.

Our broad-scale analysis will use data from the 2012 FIA database in addition to time series of classified satellite images. The FIA data will be analyzed using geographic information system (GIS) techniques to examine differences in the amount and types of habitat available to the moose population in different survey zones. We will also create a new satellite classification for portions of the moose range in NE Minnesota. This classification (based on historic and current satellite images) will be specifically developed to focus on moose habitat and will subsequently be analyzed using Fragstats and texture statistics to describe how the amount and distribution of different land cover types change across space and through time. The results of these two analyses will then be compared with the relative abundance of moose on plots with differing habitat characteristics.

To understand the process that may lead to moose selecting one landscape over the other, we need to understand how forage availability changes across space. We will characterize the forest communities in 61 sites (Figure 1) that represent a range of cover types and known disturbance histories. Our sampling methodology is adapted from previous studies in Superior and Chippewa National Forests and will help us predict how forage resources change in response to land-surface attributes (e.g., soil type, aspect, land cover). These data will allow us to determine whether coarse distributions of food and cover are correlated to local estimates of moose abundance.

Finally, to quantify how the moose population is responding to changes in the landscape, we need to describe how the spatial distribution of the animals has changed through time. The existing population estimation model was designed to provide a region-wide population estimate. We will collaborate with the MNDNR researchers to

refine the model so that it will allow for finer-grained analysis. This approach will allow us to make relative estimates of local abundance over the last 8 years. Using these results we will determine if there is spatial variation in local moose population trends and whether this variation is linked to changes in landscape characteristics.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 140,911
Amount Spent: \$ 118,328
Balance: \$ 22,583

Activity Completion Date: September 2016

Outcome	Completion Date	Budget
1. Analyze data from 1,258 FIA plots and the moose survey data to determine how broad-scale patterns of landscape change are linked to moose population dynamics.	December 2014	\$ 8,570
2. Produce a new classification of satellite data for NE MN to show how the distribution of high-quality moose habitat has changed in recent years.	September 2015	\$ 36,848
3. Identify how the species composition of moose forage changes among land-cover types and in response to stand age.	December 2015	\$ 69,448
4. Publish a spatially-explicit analysis of how moose population density changes in response to availability and arrangement of forage in the landscape.	September 2016	\$ 13,012

Activity Status as of 1 December 2014:

Data from 1,258 FIA plots has been compiled and summarized for each moose survey block. Existing land-cover data have also been summarized in those areas. We have completed an initial population analysis and will be summarizing our results in a manuscript we hope to submit early in 2015.

Activity Status as of 31 May 2015:

We have continued to analyze population data and have identified two labs at UMN to produce current and historic moose-specific land-cover data for this region. Joe Knight’s lab will lead the 2013 update and Mike Falkowski’s lab will produce historical satellite classifications so that we can compare previous populations to changes in cover type availability. We have updated the moose population data and FIA data and are continuing to develop a model that describes how the population responds to broad-scale change. Although still a preliminary analysis, we see that the areas with larger amounts of forest that also include mixtures of young birch and aspen tend to host larger local moose populations; however, broad-scale changes in landscape composition did not account for the majority of the region-wide decline. Once we have satellite classifications through time, we will be able to determine if annual changes in the structure and arrangement of the landscape is important. Our postdoc, Garrett Street, has been compiling moose location data from the DNR, Voyageurs National Park, and the Grand Portage Band to examine if changes in daily temperature during the summer affect how the animals select habitat. We are planning to compare these data to moose movement data collected in Ontario in the mid 1990s to see if there are differences in behavior across a broader range of habitats and ambient temperatures.

Activity Status as of 31 January 2016:

The production of our landcover maps is still in progress in part because Mike Falkowski left UMN for a job at Colorado State; his lab is continuing to work on the product and expect to deliver in early summer. Our 2013 map was also delayed but is nearly finished and we expect a draft in March. Despite this setback, we have been progressing well with the analysis of how moose use the landscape. The postdoc on this project, Garrett Street, has finished compiling moose movement data from a variety of sources in Minnesota and Ontario (the population in Ontario is not experiencing a decline and thus provides a useful comparison). Using a much coarser land-cover classification, we analyzed how the animals alter their habitat selection through the day and

as temperatures change during summer. We found that habitat selection patterns of moose in Minnesota were more dynamic than those in Ontario and indicated time- and temperature-dependent trade-offs between use of foraging habitat and thermal cover. Specifically, we found that during the hottest part of the day, Minnesota moose tended to choose landscapes with large amounts of treed wetlands and coniferous forest – both cover types that provide fewer and lower-quality foraging opportunities. Ontario moose tended to spend more time in mixedwood forests where thermal cover and foraging opportunities are in close proximity; this cover type is more abundant and evenly distributed in Ontario compared to Minnesota. These results suggest that differences in landscape structure may drive moose to select sub-optimal habitat when temperatures rise in the summer; this selection behavior may have long-term consequences if animals must repeatedly forgo foraging opportunities and more frequently travel between foraging habitat and thermal cover. Our next steps will be to more closely examine how variation in movement behavior is explained by broad-scale habitat characteristics.

Activity Status as of 31 May 2016:

We have received the updated classification of moose-specific cover types for NE Minnesota; production of the historical land-cover dataset is in progress and we expect to receive those classifications by August. We are now working on the analysis of how forage diversity and abundance change among cover types and disturbance histories; this analysis will be completed by the end of summer. In March, we collected winter browse across our study area and are preparing those samples for analysis. We are also preparing for our final field season in which we will focus primarily on collecting data in forested wetlands.

Activity Status as of 1 May 2017:

We have developed an improved spatially-explicit population estimation model that accounts for sightability of moose. Upon receiving the historical land-cover data on 28 April, we began calculating landscape metrics for all of the moose survey units (for time periods starting in 1999 to present). We are now starting to include metrics of land-cover arrangement and composition to see if they have an effect on local populations of moose. Our initial findings, that include only Forest Inventory and Analysis data and three years of Landsat cover-type composition data, suggest that moose populations decline slower in areas that have lower summer and winter temperatures and where there are mixtures of mature forest and young deciduous forest. We are attempting to use maps of land-cover type, LiDAR estimates of vegetation complexity, and topographic position to model forage biomass availability across moose range. This model will be important to provide context to the moose diet estimates we are developing for Activity 2 (i.e., it will allow us to determine if there is diet selection in the different areas); however, our field data show that biomass is highly variable even within cover types, so we are looking to add additional information (e.g., soils data) to improve our predictive power. To predict the proportion of time moose are travelling, foraging, and resting at different times of the day and when they are in different land-cover types, we have developed models (using observations of captive moose) to use activity data to predict behavior (our preliminary analysis is only on three animals, but these activity data are available for all animals for which we have recovered GPS collars). This analysis shows that in the summer, moose forage the most during dawn and dusk, and spend > 75% of their time resting during the middle of the day. Our next step is to link these behavioral patterns to the habitat use and diet composition of specific animals.

Final Report Summary:

I. Landscape analysis (Outcomes 1.1, 1.2)

Two separate products were created for this portion of the project. First, the Knight lab at UMN reclassified the northeastern portion of the state using the same approach they used to produce their most recent state-wide classification but with the added goal of identifying key moose habitat types. Our field plots provided additional ground-truth data for this object-based product in which land cover was classified into moose-centric cover types. This product was used for all analysis of diet and movement patterns of moose because it uses many

sources of current data (e.g., LiDAR, current ground-truth points). Because all of the supplementary data sources are not available before 2011, we needed a second approach to produce historical land-cover maps. This second product was a time series of classified imagery produced by the Falkowski lab. These data relied on historical Landsat imagery and yielded biennial land-cover maps for the study region from 1999 to 2016. This time series allowed us to identify changes in forest composition through time for the population analysis (Outcome 1.4). Both of these datasets will be made publically available on UMN DRUM in fall of 2019.

To determine how the forested landscape of northeastern Minnesota has changed in recent years, we analyzed two sources of land-cover data: the US Forest Service's Forest Inventory and Analysis (FIA) data (based on repeated surveys of forest stands from 2005 to 2015) and an 18-year, biennial time series of classified satellite data (from the Landsat satellite). We found that there has been a surprising increase in the amount of conifer forest (12% of the average moose survey unit in 1999 compared to 20% in 2015, Figure 1.1), with a decrease in mixed forest (i.e., a fine-scale mixture of deciduous and coniferous trees; 30% in 1999, 26% in 2015) and forested wetland (18% in 1999, 11% in 2015). Although the proportion of deciduous forest has stayed relatively stable since 2005 (at about 16%), the FIA data show that the composition of those stands is changing, in part due to declines in aspen, birch, and willow (key food sources for moose).

In addition to changes in the proportions of the dominant forest cover types, from 1999 to 2015 the overall landscape has become more fragmented: current landscapes have a greater edge density due to patches of different land-cover types having more complex edges (mean Edge Density increased from 8453 in 1999 to 9428 in 2015). This increase in fragmentation is subtle, but appears to be driven by decreases in the aggregation of forested wetlands and mixed forest, and increases in the edge-to-area ratios of patches of those cover types (Figure 1.2). Despite the overall increase in fragmentation, as coniferous forest has become more dominant in the region, this cover type has become more aggregated with lower edge-to-area ratios (i.e., less fragmented).

The implications of these changes on moose populations are that the landscapes in which they live are being altered in ways that may be suboptimal for their needs. While all land-cover types are used by moose in some fashion, the relative amounts of these cover types, their distribution in the landscape, and the plant composition of their understory (i.e., the quality and abundance of moose food) will affect where moose go and how well they are able to survive and reproduce when they get there.

II. Forage availability (Outcome 1.3)

a) Forage availability by cover type

We estimated forage availability at 70 sites distributed within three broad areas of moose range (Figure 1.3). These areas were distinguished by their summer temperatures (Figure 1.4) and are referred to as Cold (NE portion of moose range near Grand Marais), Moderate (SE portion of moose range near Isabella), and Warm (NW portion of moose range near Ely). We classified potential food plants into four different groups based on previous studies of moose dietary preference: aquatics, high, medium, and low. The species that we categorized into each group are as follows:

- o aquatics – all aquatics collected from the warm and cold regions
- o high – paper birch, trembling aspen, and all willows.
- o medium – all cherries and maples, as well as mountain ash
- o low – all species of service berry, dogwood, and alder, in addition to balsam fir, beaked hazel, and any other species that are not included in the original preference study but might be encountered and sampled on an occasional basis (e.g., green and black ash, red pine, and white pine)

Using standardized methods developed by the UMN Department of Forest Research, we recorded the species composition and biomass of each of the forage preference groups. We found that all forest types had greater amounts of high-preference forage in the Cold region, while the Warm and Moderate region forests had more low-preference forage (Figure 1.5).

Collectively, our results suggest that cover type plays an important role in dictating the availability of forage for moose, with mixed and coniferous forest types offering more forage than any other cover type (Table 1.1). When compared to the amount of forage available in conifer habitat, grasslands and regenerating forest had substantially less food available to moose. Although we also found statistical evidence that deciduous and wetland habitat offer less forage for moose, the support for this relationship was not as strong (Table 1.1). We were initially surprised that regenerating forests tended to have much lower forage availability than other cover types; however, the reason for this is that regenerating forests are highly variable in biomass and species composition depending on the age of the regeneration (e.g., one year after a severe fire would have different forage availability than 10 years after a fire).

Overall, our results emphasize the importance of mixed-wood forest, which tended to have as much or more forage biomass than coniferous stands. It is also important to point out that while forage from different preference groups also varied among cover types, these differences were strongest for low and medium-preference forage (Table 1.2). Evidence for differences in the availability of high-preference forage among cover types was marginal.

Table 1.1 Results of generalized linear model testing the influence of cover type on overall forage availability. We used coniferous forest as our reference category.

cover type	estimate	standard error	t-value	p-value
deciduous forest	-0.3780	0.1868	-2.023	0.0472*
wetland	-0.3779	0.1678	-2.252	0.0278*
grassland	-0.6894	0.1647	-4.185	0.0001*
mixed forest	0.1745	0.1715	1.018	0.3126
regenerating forest	-0.6147	0.1596	-3.851	0.0003*

*Indicates a statistically significant difference ($\alpha = 0.05$) when compared to our reference group (coniferous forest).

Table 1.2 Results of one-way ANOVAs assessing how forage from different preference groups varies among cover types. For all tests we used coniferous forest as our reference cover type.

preference group	cover type	estimate	standard error	t-value	p-value
low	deciduous forest	-0.20543	0.07876	-2.608	0.0113*
	wetland	-0.11318	0.07075	-1.600	0.1146
	grassland	-0.35465	0.06944	-5.107	< 0.0001*
	mixed forest	0.11146	0.07227	1.542	0.1279
	regenerating forest	-0.29696	0.06728	-4.414	< 0.0001*
medium	deciduous forest	-0.03562	0.03582	-0.994	0.3238
	wetland	-0.10010	0.03218	-3.111	0.0029*
	grassland	-0.10826	0.03158	-3.428	0.0011*
	mixed forest	-0.03089	0.03287	-0.940	0.3508
	regenerating forest	-0.10483	0.03060	-3.426	0.0011*
high	deciduous forest	-0.13694	0.12419	-1.103	0.2743
	wetland	-0.16466	0.11157	-1.476	0.1449
	grassland	-0.22644	0.10950	-2.068	0.0427*

mixed forest	0.09395	0.11397	0.824	0.4128
regenerating forest	-0.21289	0.10610	-2.007	0.0490*

*Indicates a statistically significant difference ($\alpha = 0.05$) when compared to our reference group (coniferous forest).

b) Forage availability by stand age

The 70 semi-permanent plots that we sampled represented disturbed stands from three different time periods (2002, 2006, and 2011) in addition to areas that have not experienced any known disturbance in the recent past (≥ 25 years). For each of the stand ages, we calculated the mean biomass (kg/m^2) of each forage preference group across different stand ages (≥ 25 years, 13 years, 9 years, and 4 years) to estimate how biomass availability changes with disturbance in each temperature region (Figure 1.6). There was no significant difference with respect to disturbance age for any class except the medium-preference forage (there was slightly more of this food source in the 13-year old stands); however, the warm and moderate regions had consistently lower biomass in all preference groups and stand ages, compared to the cold region (Figure 1.6).

We used generalized linear models to determine if the overall availability of forage varies as a function of stand age and multivariate analysis of variance (MANOVA) to determine if the availability of forage from different preference groups varies as a function of stand age. For MANOVA tests that had a significant effect, we performed one-way ANOVA's to determine the most important relationships.

The influence of stand age on forage availability varied among age categories (Table 1.3). Specifically, overall availability of forage in 9-year old stands is significantly lower from that available in control plots (stand age ≥ 25 years), whereas there was no difference between control plots and stands that were either 4-years old or 13-years old. However, despite this lack of significance, the disturbed stands typically had less biomass than the control plots (Table 1.3). The relative proportions of different forage preference groups was highly variable, but the moderate and warm regions were more similar in their distributions than the cold region (Figure 1.5).

Table 1.3 Results of generalized linear model testing the influence of stand age on overall forage availability. We used forest stands with an age of 25 years or more as our reference category.

Stand Age	estimate	standard error	t-value	p-value
4 years	-0.3587	0.2129	-1.934	0.0575
9 years	-0.4587	0.2150	-2.134	0.0367*
13 years	-0.4117	0.2092	-1.714	0.0912

*Indicates a statistically significant difference ($\alpha = 0.05$) when compared to our reference category.

III. Moose Population Density (Outcome 1.4)

We examined how moose were distributed across the region by quantifying, 1) what landscape and environmental factors affect moose movement and habitat selection decisions, and 2) how broad-scale characteristics of the landscape (e.g., the composition of forest cover types within each moose survey unit) affect the local population growth of moose. To describe the individual-scale patterns of moose habitat use, we examined how moose in Minnesota changed their movement patterns in response to available habitat and ambient temperature. We then compared these patterns to those from moose in Ontario, Canada. We estimated summer resource selection models for 134 adult female moose in Minnesota and 64 in Ontario. We

found that while the moose in Ontario did not show strong patterns of resource selection (and very little response to ambient temperature), the Minnesota moose had strong patterns in selection that changed both throughout the day and in response to temperature (Figure 1.7). In particular, MN moose selected for mixed forests and treed wetland during the middle of the day and whenever the temperature was above their thermal optimum (i.e., 14° C). In the evening hours, or during lower temperature periods, the moose tended to favor open uplands. Overall, moose used the mixed forest type much more than expected based on availability throughout the day. This suggests that it is a critical habitat for moose, likely because of the large amount and diversity of forage available (see II above) and because of the fine-scale mixture of coniferous and deciduous trees that allows for thermal cover during the hottest parts of the day. The contrast with moose from Ontario was marked; however, this difference is likely because the mixed forest type dominates the Ontario landscape, so moose have an abundance of thermal cover and food and thus do not need to alter their foraging or movement patterns in response to temperature. These results were published in *Landscape Ecology*: Street et al. 2016, and an advance in statistical methodology that stemmed from this research was published in *Ecography*: Fieberg et al. 2017.

To determine what factors might be linked to changes in moose population density across the range, we developed a spatially explicit population model that used raw data from the DNR moose survey to estimate differences in population growth rate. After using moose resource selection patterns during the survey period to calibrate sightability, we found that moose populations were greater in areas with more mixed and young forest but less deciduous forest and open water (Figure 1.8). Populations were also greater in areas with larger patches of regenerating forest, smaller patches of coniferous forest, and less fragmented forested wetlands. Finally, moose populations did worse in areas and years where there were higher than usual summer temperatures (i.e., the heat stress index was greater). Clear patterns of high and low populations emerge across moose range, but they also change through time (Figure 1.9). When the predicted number of moose per survey unit was summed, the range-wide population estimate of this model is very similar to that produced for the region by the MN DNR (Figure 1.10). The advantage of our spatially explicit model is that it allows researchers and managers to more closely examine areas that are either doing well or declining in numbers and then use what is learned there to develop targeted interventions.

This model explains how spatial and temporal variability in temperature and land cover can directly affect moose populations; however, despite the influential patterns we have discovered, it is important to note that there was a large, unexplained annual effect that was not directly linked to the factors we measured. Further, some factors may have important interactions with each other, may only be correlated with true drivers of population dynamics, or have effects that operate on a time lag greater than one year. For example, temperature alone (here included as summer Heat Stress Index, or the cumulative number of degrees that exceed 14°C during the summer) cannot explain the decline of the moose population by itself. This index was actually higher for more years in the late 1980s (Figure 1.11), and while there are no reliable moose population estimates from that time period, anecdotal evidence does not support a previous decline in this region. This model should be used to develop large-scale experimental manipulations in moose range to determine how altering the pattern of forest patches can affect usage by moose (and subsequent effects on the local population size). Future work with this model will involve collaborations with researchers currently monitoring moose habitat restoration areas and land managers of state, federal, private, and tribal land. The goal will be to initiate long-scale manipulations that will be monitored for long time periods (10-20 years).

Figures (Activity 1)

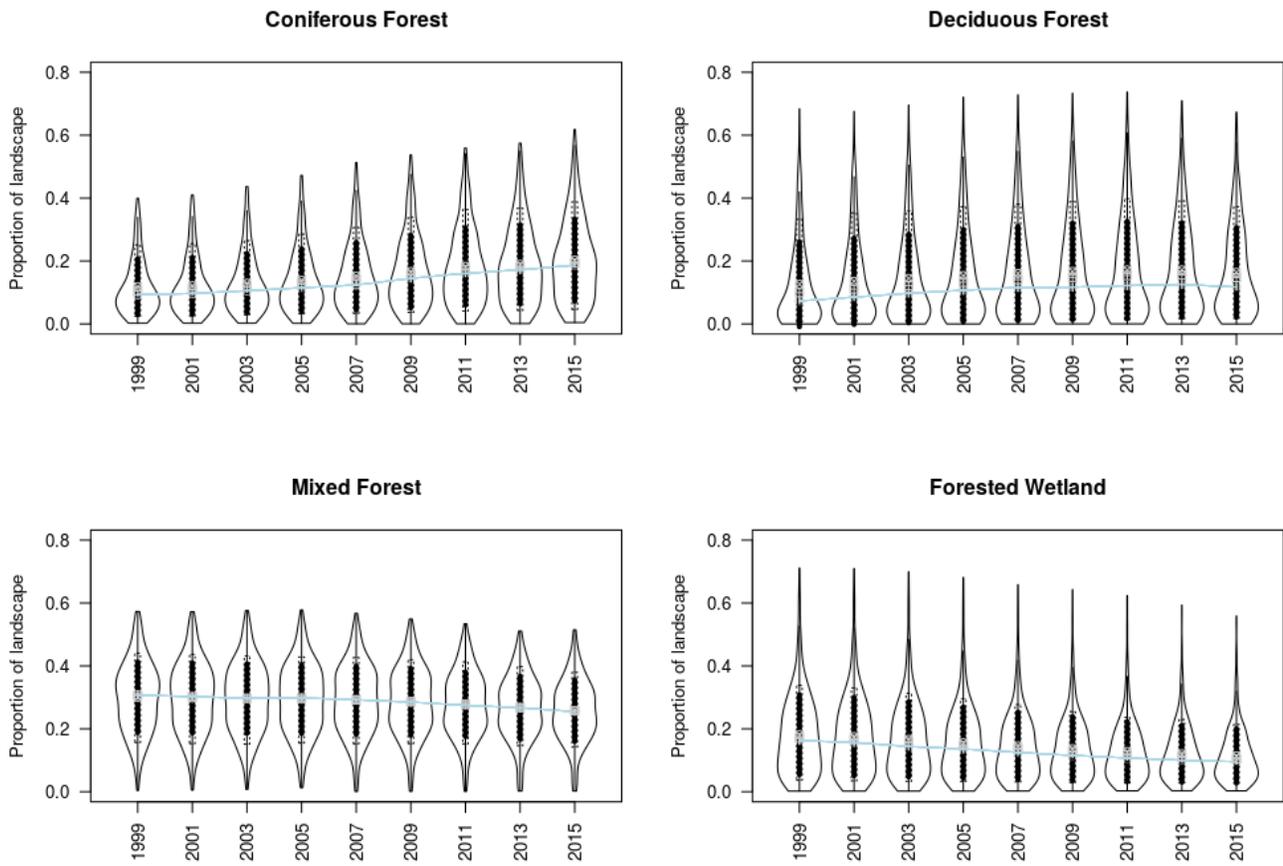


Figure 1.1 Violin plots of the proportion of dominant forest types in moose survey units from 1999 to 2015.

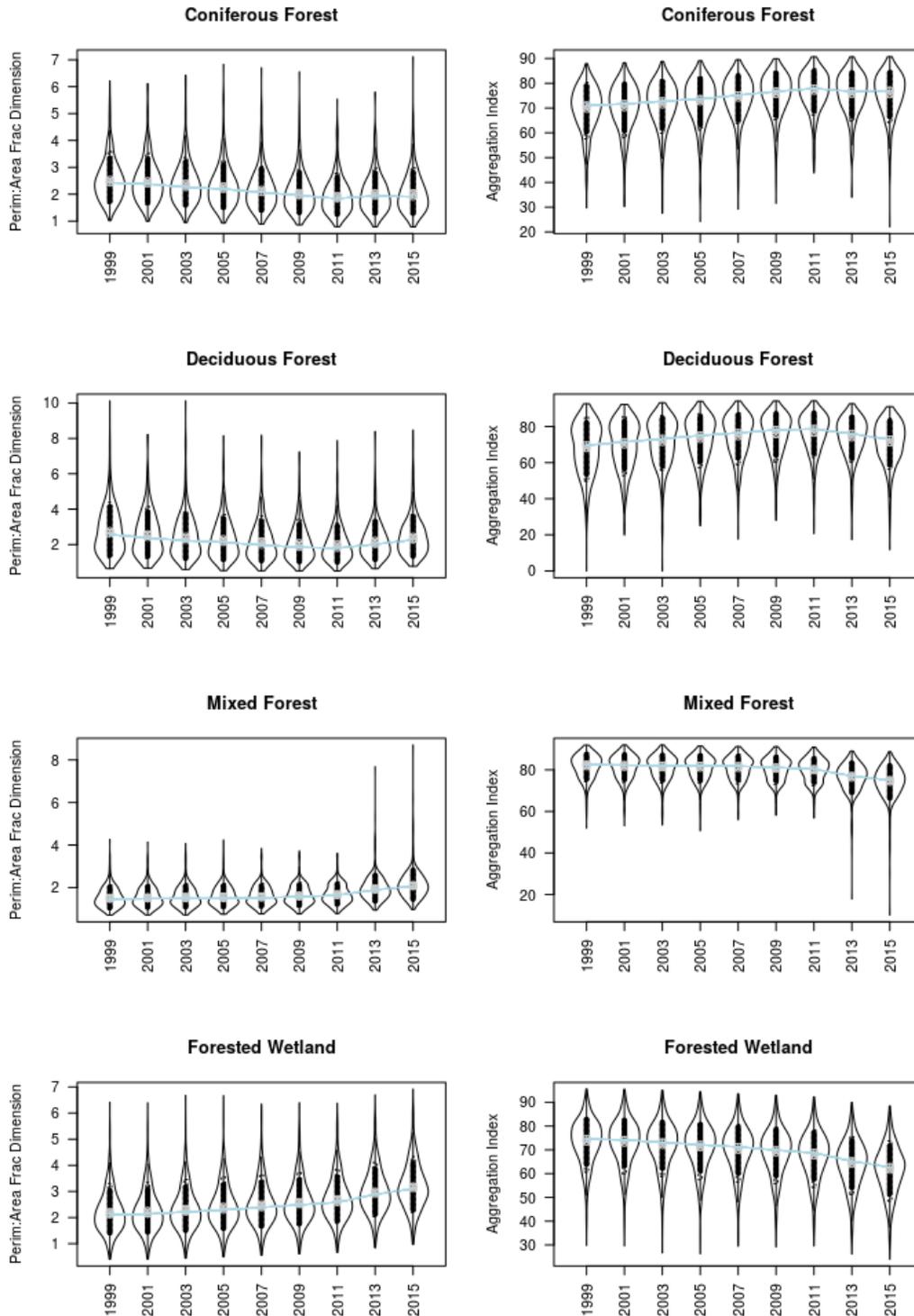


Figure 1.2 Two measures of fragmentation calculated for the major forested cover types. Larger values of the Perimeter:Area Fractal Dimension metric indicate that patches of that cover type tended to have more complex edges (the smallest possible value of 1 would indicate a square patch). Larger values of the Aggregation Index indicate that the cover type in question tends to be in fewer numbers of tightly packed patches in the landscape. Collectively, these figures show that Conifer forests have become less fragmented while the other cover types, especially Forested Wetlands and Mixed Forest, have become more fragmented.

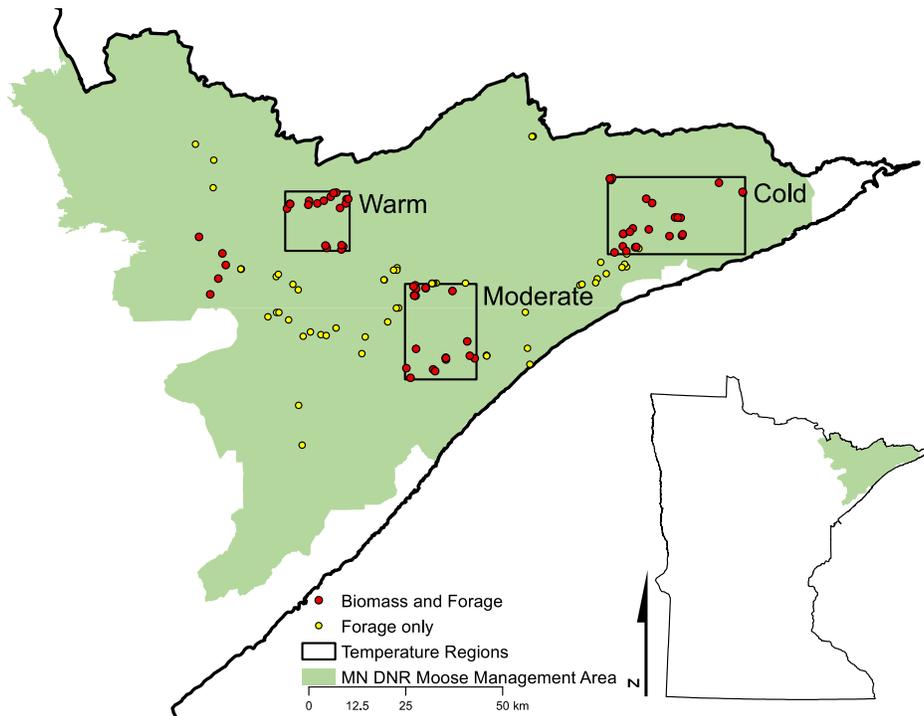


Figure 1.3 *Distribution of biomass plots, forage sampling plots, and designated temperature regions across northeastern Minnesota. Biomass data and forage samples for stable isotope analysis were collected at those locations identified as “Biomass and Forage.” Sites identified as “Forage only” were visited for the sole purpose of collecting forage samples.*

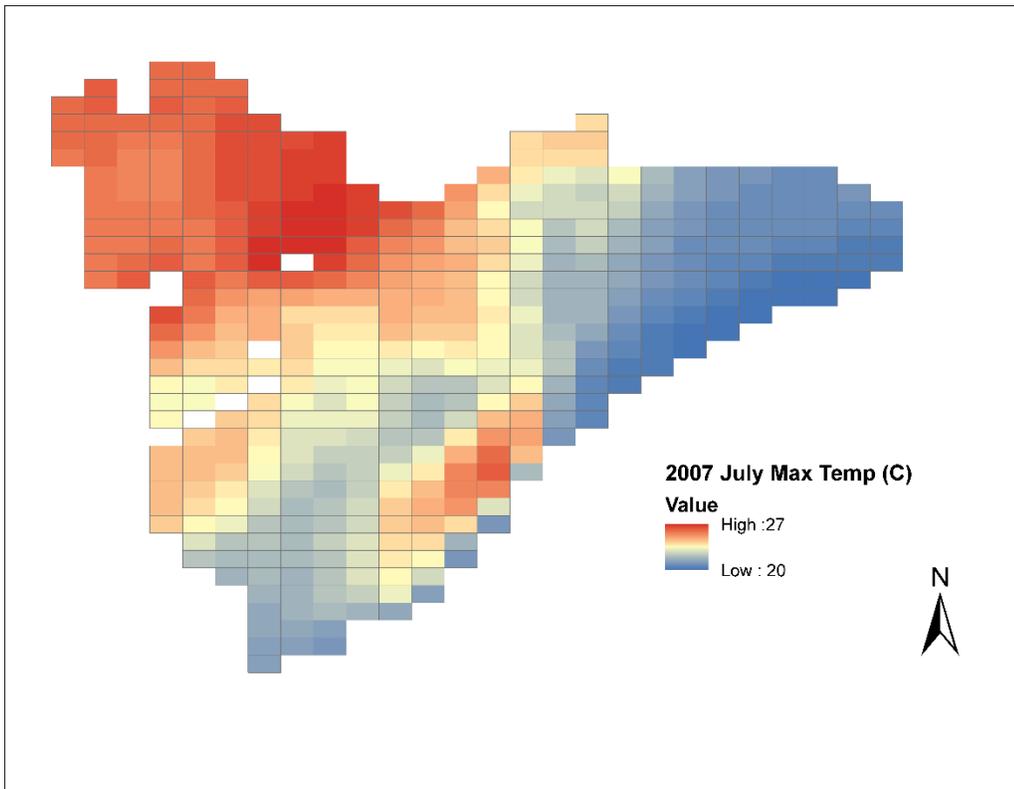


Figure 1.4 Mean maximum July temperatures in survey blocks across moose range in 2007. Temperature data are from the PRISM data set.

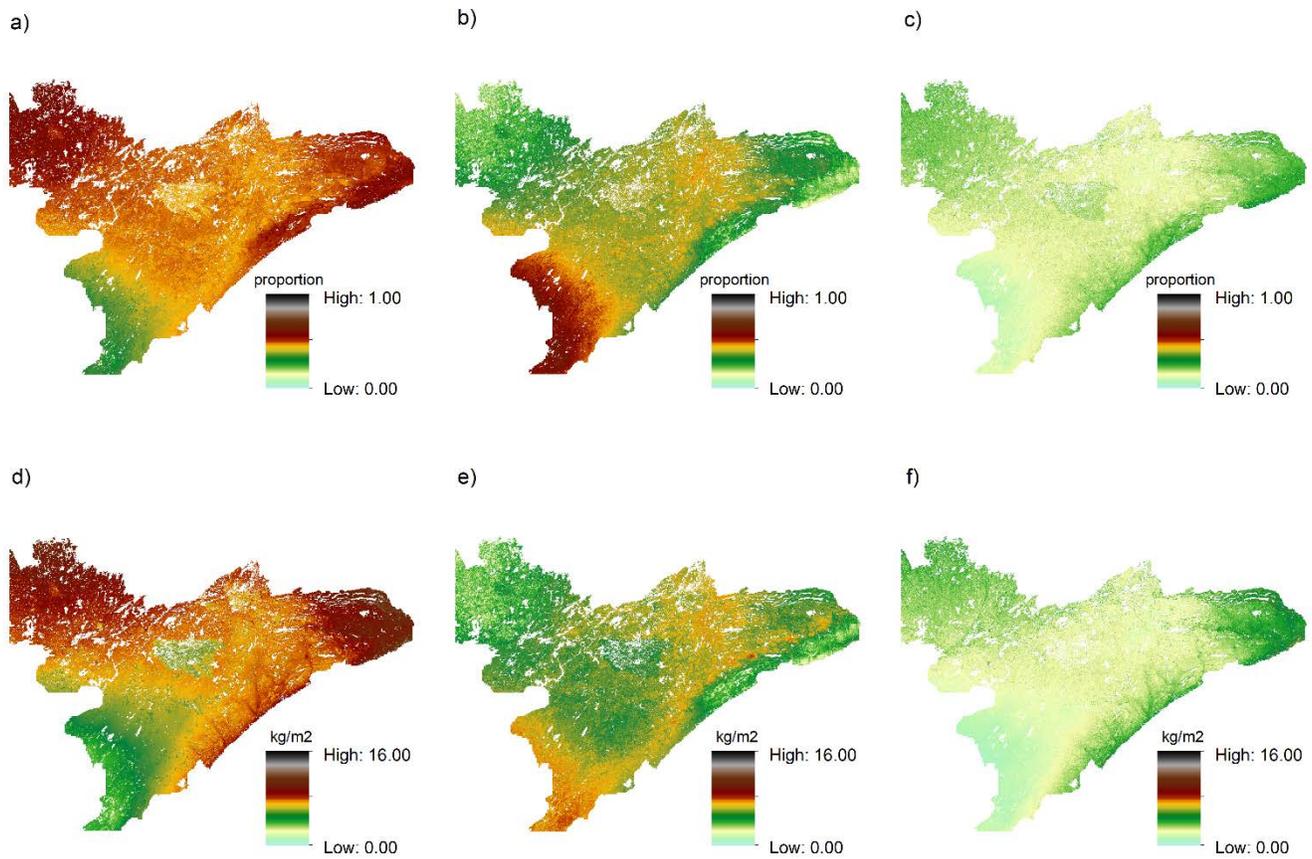


Figure 1.5 Maps of the relative abundance (a, b, c) and estimated biomass (d, e, f) of the three preference groups of terrestrial forage: Low (a, d), Medium (b, e), and High (c, f). Low-preference forage is present and abundant throughout the study area, but High-preference forage is much more prevalent in the northeastern portion of moose range.

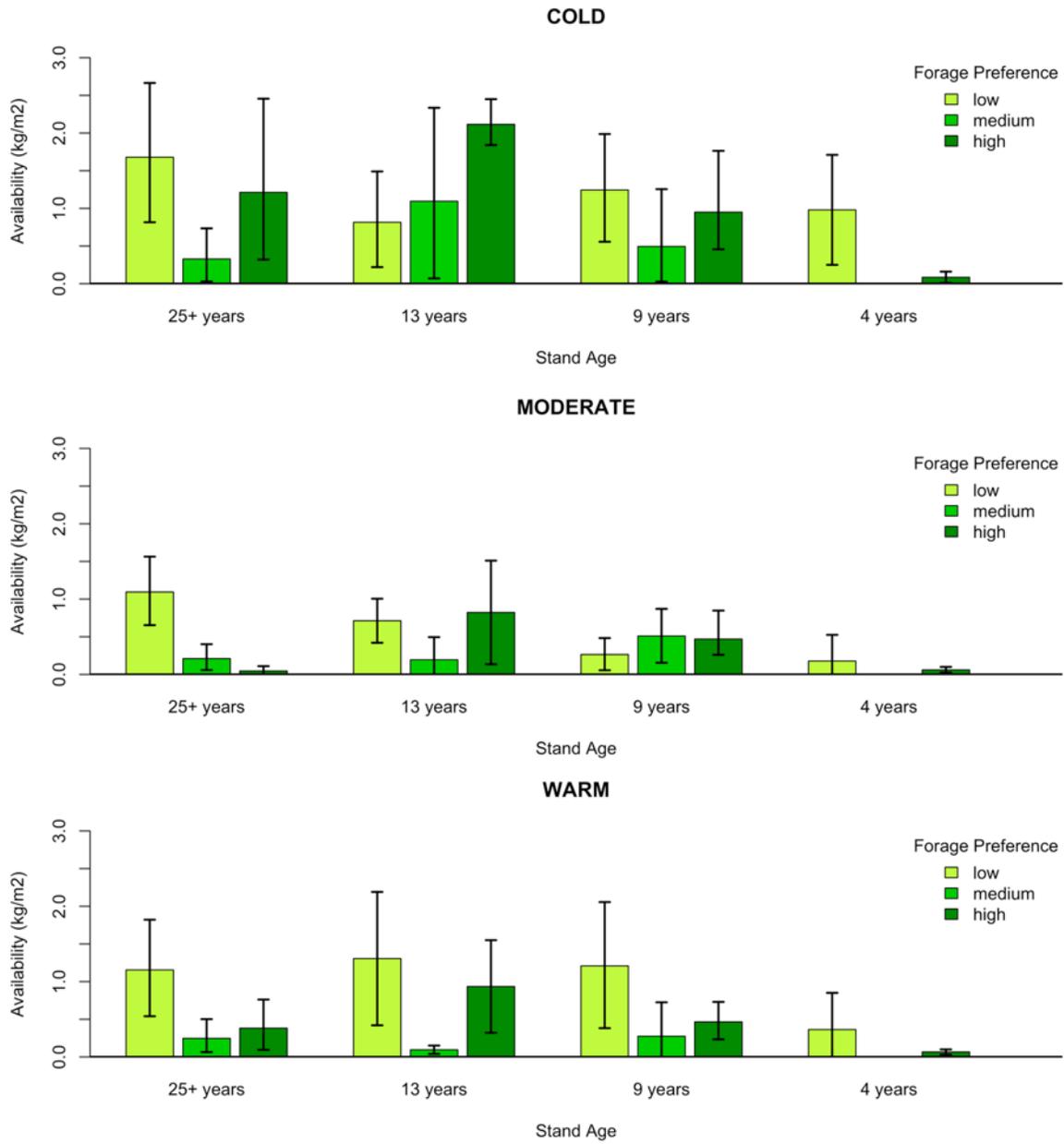


Figure 1.6 Availability of different forage preference groups (low, medium, high) within each temperature region, as a function of stand age. Lines represent 95% bootstrapped confidence intervals.

Minnesota

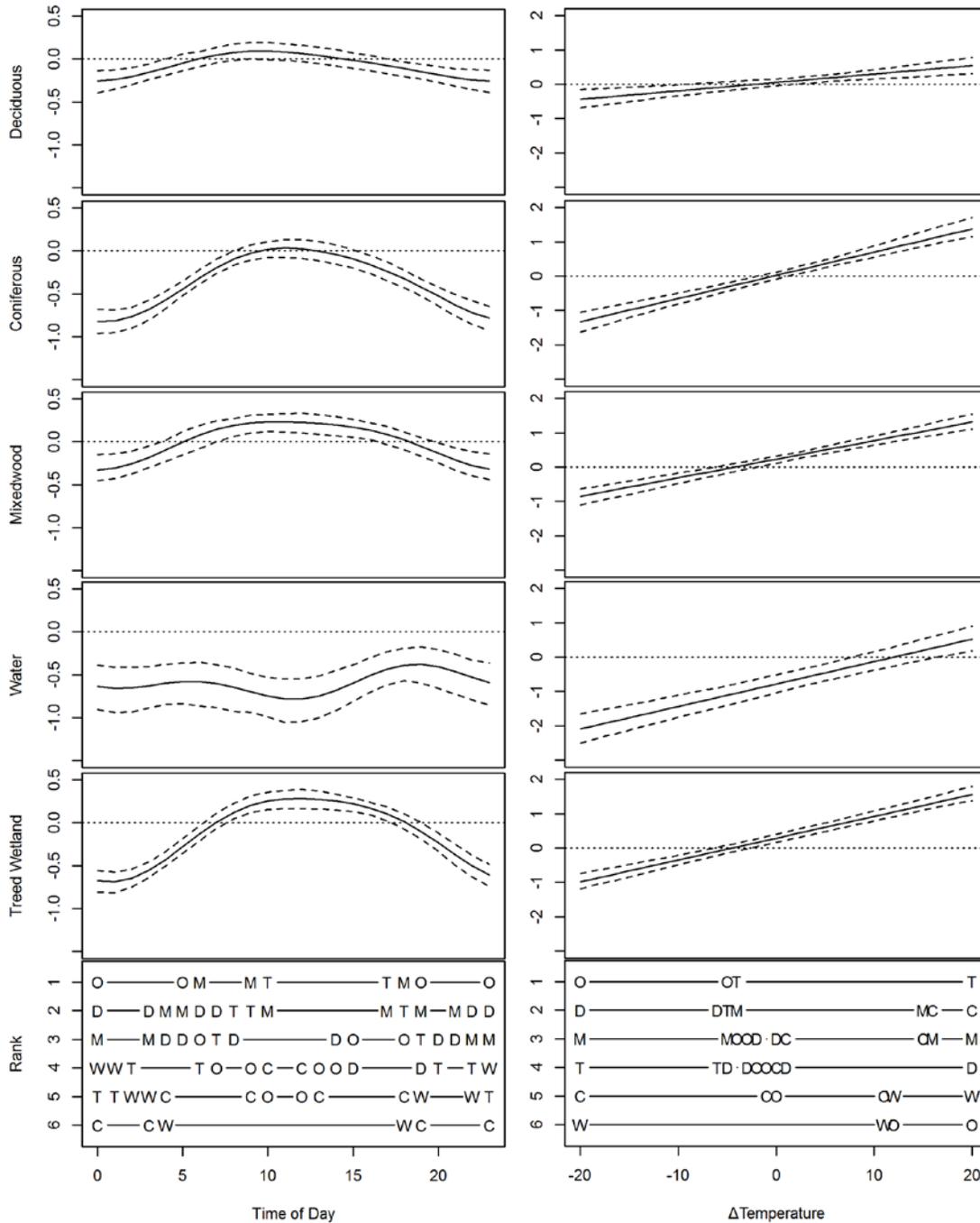


Figure 1.7 Predicted selection strength (log relative risk, solid lines) by moose with 95 % Confidence Intervals dashed lines) for 100 % cover by land cover classifications during summer (June 1–September 30) in Minnesota across both time of day (left column) and temperature (C) scaled to moose upper thermal optima (right column). Temperature is held constant at the moose upper thermal optimum (i.e., Δ Temperature = 0° C) in time of day plots, and time is held constant at noon in Δ Temperature plots. Bottom panels indicate relative rank of selection strength for each land cover class (D deciduous; C

coniferous; M mixed forest; W water; T treed wetland; O other) across the diurnal cycle and temperature gradient. Figure from Street et al. (2016).

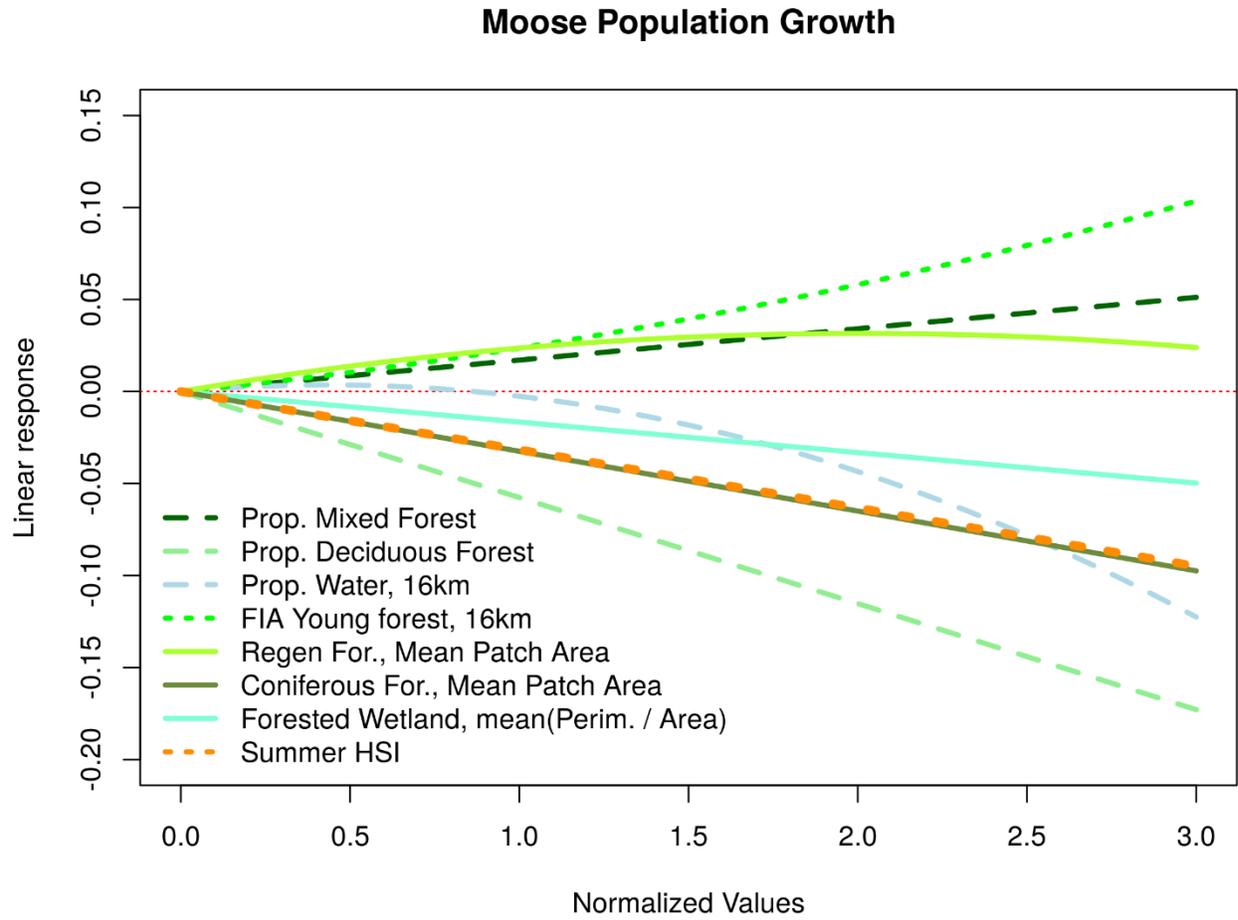


Figure 1.7 *The effect of different factors, calculated at the level of moose survey units, on the population growth rate of moose. All factors are normalized to allow for direct comparison of their effects.*

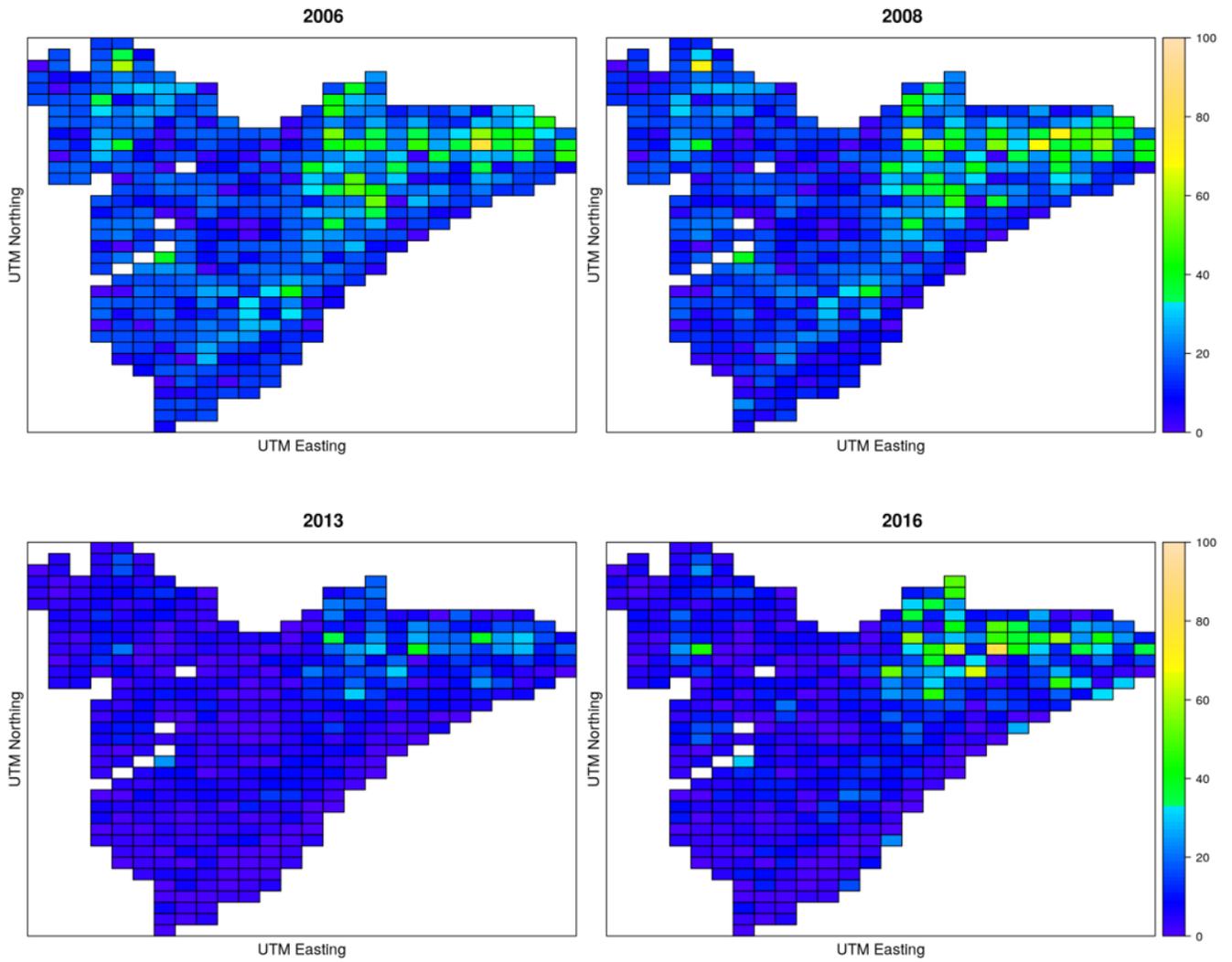


Figure 1.9 Spatially explicit estimates of the Minnesota moose population through time. Rectangles are moose survey units established by MN DNR.

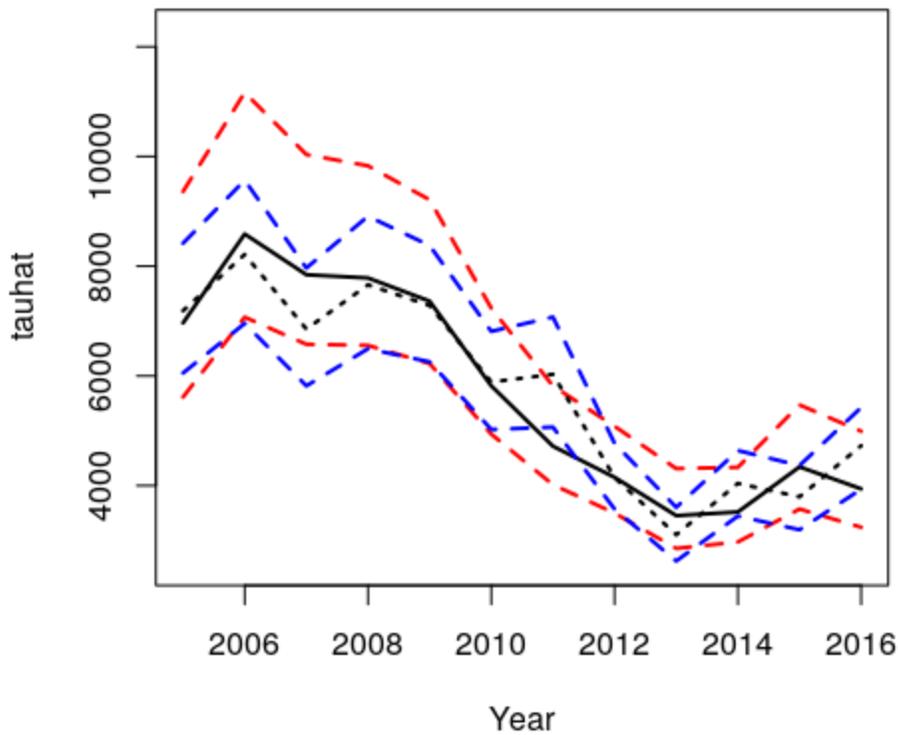


Figure 1.10 Region-wide population estimations through time. The solid black line is the MN DNR estimate based on an established sightability model (red lines are 90% prediction intervals). The dotted black line is the population trajectory estimated from the spatially explicit model developed here (blue lines are 90 % prediction intervals).

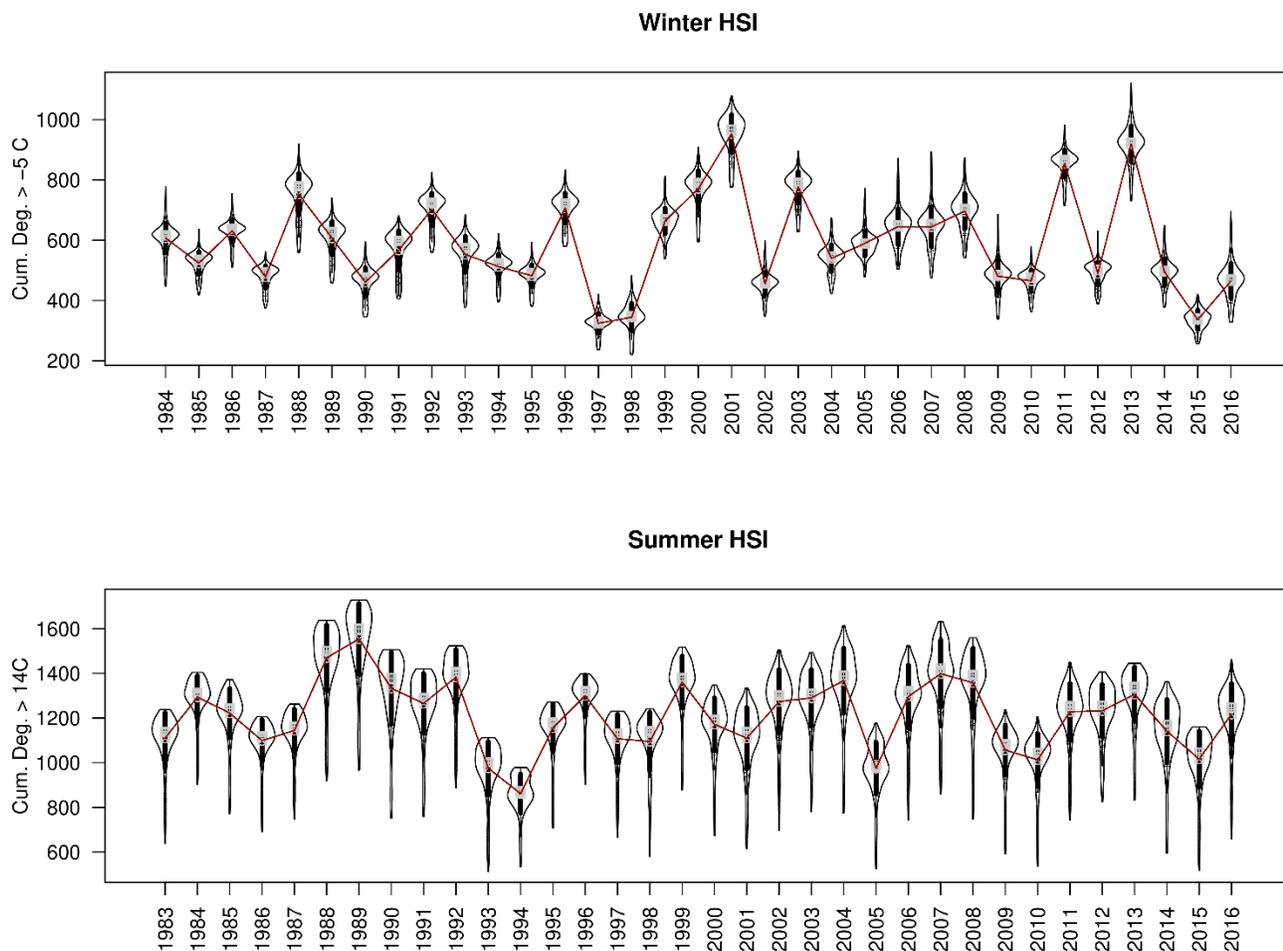


Figure 1.11 Violin plots of Heat Stress Index for Summer (top) and Winter using data collected within moose survey units from 1984 to 2016.

ACTIVITY 2: Linking the distribution and quality of food and cover to moose diet, body condition and mortality risk.

Description: We will use stable isotope analysis to determine how the distribution of food and cover affects diet and whether individual movement behavior allows some individuals to have higher quality diets in landscapes with lower quality habitat. We hypothesize that diets of individual animals will reflect the forage available to them within their home range area and that animals that live in areas with lower quality forage or larger distances between food and cover will have lower body condition and be more susceptible to mortality. By analyzing the carbon and nitrogen isotopic ratios of moose body tissues collected at capture and after death, we can assess individual moose diet and habitat use on timescales from several weeks to several years. We will combine these data with GPS locations of the same animals to test if the moose are eating what is available to them. This will allow us to determine the degree to which landscape context (e.g., the abundance, spatial distribution, and biochemical signature of land-cover types within an animal's home range) is driving the movement pattern and diet of the animal. We will then determine if dietary differences among individuals can explain variation in mid-winter body condition or mortality risk. These results will provide suggestions on how to change forest management to benefit moose.

During Years 1 and 2, we plan four field sessions of unequal duration each utilizing two field teams: (1) in an early spring session we will sample leaves and wood of common forage in one replicate plot of each land-cover type; (2) in a late spring session, we will revisit the same sites to describe early phenological changes in vegetation quality and isotopic composition; (3) in a longer summer session, we will focus on the entire range and sample leaves, wood, and fruiting bodies in three replicates of each land-cover type; (4) a winter session will focus on woody forage in one replicate of each treatment. As field conditions allow, the winter plots will be the same as those sampled in spring, ensuring seasonal sampling of the same plots over two years, and in each of these plots we will mark specific plants for replicate sampling. This sampling scheme will control for seasonal and inter-annual variation in forage composition over the course of the project. In Years 2 and 3 we will use the movement data collected from the GPS collars to ensure that we sample plants within known home ranges; this may require establishing some new plots. During winter sampling in Years 2 and 3, we will backtrack moose paths known from collar data to sample consumed vegetation and collect snow urine. Given the number of plots and samples planned, flexibility in sampling during Years 2 and 3 is possible and will allow us to concentrate on known home ranges without sacrificing the comprehensiveness of sampling. Year 3 will also include revisits of a subset of sites and marked plants (this year will also include substantial ground truthing efforts for the satellite classifications).

The stable isotopic composition of vegetation sampled in the field will be related to that of moose tissues we collected at capture. To develop robust estimates of diet, we need to analyze a large number (7368) of individual plant and animal tissue samples. For the moose, we will primarily focus on hair and hoof keratin, although we will opportunistically sample feces, bone, and tooth enamel from dead animals. By sampling moose tissues with different elemental turnover times that integrate diet over different intervals and for which isotope enrichments relative to diet are known, we can assess individual moose diet and habitat use on timescales from days to months to years.

We will use statistical models to describe the survival for adult moose as a function of animal characteristics (e.g., age, sex, behavioral phenotype, short- and long-term diet based on stable isotope analysis, etc.) and landscape covariates (e.g., road density, land cover proportions, land cover patch metrics, etc.) calculated within each animal's home range. We will then use these results to develop spatially explicit risk maps that we can compare to the local moose population trajectories developed in Activity 1. Combining these two sources of data will help us understand if the distribution of food and cover are mechanistically linked to the population dynamics of moose in Northern Minnesota. The results from this analysis will allow us to make specific

management recommendations related to the distribution and abundance of different land-cover types that will increase the probability of stabilizing the moose population.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 159,089
Amount Spent: \$ 81,606
Balance: \$ 77,483

Activity Completion Date: *June 2017*

Outcome	Completion Date	Budget
1. Assess the nutrient quality and stable isotopic concentration of forage available in each collared animal's home range.	November 2015	\$118,413
2. Develop a time series of diet over the previous year for each collared moose (n=129) using stable isotopic analysis of hair collected at capture and after death.	December 2015	\$15,736
3. Assess whether forage availability or diet affect the rates of survival.	December 2016	\$33,172
4. Provide specific forest management recommendations to experimentally improve the landscape for moose in the areas of their range where the animals are most vulnerable.	June 2017	\$4,801

Status as of 2 December 2014:

During summer of 2014 we sampled vegetation at roughly 140 sites across northeastern Minnesota, and collected more than 2500 plant samples across 8 different species, ranging from low to high preference for moose. At each of these sites, we also estimated browse diversity and are currently working on estimating forage availability throughout the geographic range of moose in northeast Minnesota.

To date, we have logged all plant samples with a unique identification number and are currently preparing to strategically analyze forage samples for stable isotopes of carbon and nitrogen. Currently we have roughly 100 plant samples that are ready for stable isotope analysis. By mid December, we will have an additional 40+ aquatic vegetation samples prepared for stable isotope analysis. In the past month, we have run stable isotope analysis on more than 250 moose hair samples.

Activity Status as of 31 May 2015:

The mass spectrometer that we use has been unavailable for the early part of this year; however, we are beginning to run samples again as of 1 May. Using the data we have thus far, we began an exploratory analysis focused on determining whether there is a strong spatial pattern in the stable isotope composition of a key forage species (paper birch) across moose range. This is critical to understand because our goal is to compare the isotopic composition of forage plants to that of moose hair and thus estimate the likely diet of individual animals. Working with an undergraduate UROP student at UMN, we have found that the isotope values of paper birch do vary in a predictable manner based on disturbance history and region. Although our estimates of crude protein (one measure of forage quality) present in the samples were constant across the study region, we found that the carbon and nitrogen isotope ratios of paper birch both increased in stands recently disturbed by wildfire or timber harvest. Further, the carbon ratios and nitrogen ratios increased and decreased respectively in the northwestern portion of the study area (compared to the northeast). Despite this broad-scale effect, there does not seem to be a strong fine-scale pattern to account for other than the impact of disturbance history; we will conduct similar tests with other forage species as the data come in from the mass spectrometer. When examining isotope ratios of moose hair, we see similar patterns: the nitrogen isotope ratios increase from North to South, while the carbon isotope ratios increase from East to West. These trends may be a function of regional changes in the isotopic composition of forage (as observed in paper birch), but they may also result from differences in what is available and palatable to the animals. Our next steps will be to attempt to tease apart these effects.

Our study region includes areas that experience very different summer temperatures (a difference in mean summer temperature of approximately a +6 degrees Celsius from Grand Portage to Ely). Temperature may affect the secondary compounds produced by plants to reduce palatability and digestibility of the plant tissue by herbivores (i.e., an overall reduction in effective forage quality). To understand how this may affect moose forage, we sampled trees grown in the B4Warmed study to experimentally test whether warmer temperatures during the growing season lead to different chemical compositions of paper birch and balsam fir. We will be collecting field samples of these species at our study plots this summer to see if we can detect region-wide and land-cover specific differences in the impact of summer temperature on forage quality.

Activity Status as of 31 January 2016:

The mass spectrometer malfunctioned over the summer and has been out of commission for a number of months. We are only now starting to get samples run; however, at this point we have first priority in the queue and expect to have our samples completed by November 2016. Although this is somewhat later than expected, we do not have other options and are still making progress on the project. We are currently revising a manuscript (reviewed in the Journal of Ecology) about how ambient temperature affects the chemical composition of moose forage species (specifically paper birch and balsam fir). We found several important results in an experimental setting. As temperatures increased: 1) the diversity and relative abundance of secondary compounds changed for both species; 2) balsam fir reduced the total number of compounds produced and paper birch reduced variance in their abundance; and 3) the concentrations of two representative compounds, catechin and diterpene resin acid, both declined. These results suggest that we may see changes in the relative palatability of different forage species across the landscape; in the coming months we will be testing samples collected from our study sites to see if the trends we observed in the experimental plots hold up in the field. As we get more stable isotope data from our plant samples, we will be able to develop diet models for individual animals to see if the moose are eating different plants across the region (and if this is linked to changes in the abundance).

Activity Status as of 31 May 2016:

We conducted a brief winter field season to collect winter forage from many of our sites across NE MN. These samples will be used to answer the question of how winter forage quality changes (if at all) across moose range. Our stable isotope data continue to come in, but we have been developing a workflow for analysis so that once all of the plant tissue samples are analyzed we can finalize our statistical results quickly. Using the data we have collected thus far, our preliminary results suggest that the composition of moose diets change both across space and through time. For example, in the central part of the range, moose diets consist of roughly 9% paper birch (a high-preference food) during spring; whereas in the fall, the composition of paper birch in the diet increases to about 30%. Similarly, diets in the western part of the range are comprised of about 21% paper birch in the spring, and about 43% in the fall. However, the use of balsam fir (a low-preference food) remains relatively constant across the geographic range of moose in Minnesota, regardless of season, making up roughly 2% to 5% of the diet. Moose diets in the eastern-most part of the range do not appear to change with season. We have also begun to analyze hair collected postmortem from collared moose. This will allow us to determine if diets of individual moose change dramatically year to year, and whether knowing the animals' movement patterns help us to better predict their diet.

Activity Status as of 1 May 2017:

The mass spectrometer in the Fox lab again malfunctioned during summer 2016 and has been out of commission since. Starting in January 2017, we began sending forage samples to another lab for analysis of stable isotope composition. To date, we have analyzed more than 900 forage samples analyzed from 11

different species and 147 individual moose for stable isotopes of carbon and nitrogen, with plans to analyze another 2000 plant samples by the end of May. Preliminary analyses suggest that early summer diet varies throughout the geographic range of moose in Minnesota, and this variation is correlated with mean summer temperature. In the coldest parts of their summer range (close to Grand Portage), more than 80% of ingested forage during early summer consists of only two species – willow (44%) and maple (42%), while in the warmest parts of their summer range (close to Ely), 80% of ingested forage during early summer consisted of five different species – willow (19%), beaked hazel (17%), trembling aspen (16%), paper birch (14%), and juneberry (11%). These results suggest moose in the coldest part of their range have much lower dietary diversity than moose in the warmest parts of their range. Moreover, these changes do not appear to be associated with differences in the availability of different forage species, suggesting that moose in the coldest parts of their range are more selective feeders than moose in the warmest parts of their range. We are starting to analyze data collected from 100 temperature loggers that have been intermittently recording temperature at two-hour intervals since 2012 throughout the geographic range of moose in northeastern Minnesota. Preliminary analysis of these data suggests that during our study, 2013 was both the coldest and hottest year for moose in Minnesota. During summer 2013, some recorded temperatures exceeded 100°F and during winter of that same year, temperatures dropped to as low as -42°F. By the end of May, we will have data that will allow us to determine if ambient temperature and/ or land cover are influencing the chemical composition of forage in a way that alters palatability of different forage species, thereby influencing the diet composition estimates noted above.

Final Report Summary:

IV. Forage Quality and Forage Isotopes (Outcome 2.1)

a) Quality of Forage Plants

We collected plant samples at 131 sites (Figure 1.3) and found that plant species considered to be highly preferred by moose were indeed of higher quality, based on Carbon:Nitrogen ratios (C:N; lower is better) and %Nitrogen (%N; higher is better). The quality of forage varied spatially across moose range, with the area currently supporting the highest populations of moose (i.e., NW of Grand Marais) having the best combinations of C:N and %N (Figures 2.1 & 2.2); it is important to note that this area also has the highest forage biomass of all regions (Figure 1.5).

Because of the strong gradient of ambient temperature seen across moose range (Figure 1.4), we also tested whether plant chemistry changed in response to temperature. Specifically, we examined how ambient temperature and canopy cover affected the production of plant secondary metabolites (PSMs), which include chemical defenses produced by plants (i.e., chemicals that could cause a moose to avoid an otherwise high-quality plant). We compared common high and low quality forage plants in the B4Warmed experimental plots and also collected plant material from across the study area to explore how landscape-scale variation of abiotic conditions could impact the PSM profile of important forage plants.

Plant secondary metabolites are a key mechanism by which plants defend themselves against potential threats, and changes in the abiotic environment can alter the diversity and abundance of PSMs. While the number of studies investigating the effects of abiotic factors on PSM production is growing, we currently have a limited understanding of how combinations of factors may influence PSM production. The objective of this portion of our study was to determine how ambient temperature influences PSM production and how the addition of other factors may modulate this effect. We used untargeted metabolomics to evaluate how PSM production in five different woody plant species in northern Minnesota are influenced by varying combinations of temperature, moisture, and light in both experimental and natural conditions. We used perMANOVA to compare PSM profiles and phytochemical turnover across treatments and NMDS to visualize treatment-specific

changes in PSM profiles. Finally, we used linear mixed-effects models to examine changes in phytochemical richness.

Under closed-canopy, experimental warming led to distinct PSM profiles and induced phytochemical turnover in paper birch but not balsam fir (Figure 2.3). In open-canopy sites, warming had no influence on PSM production (Figure 2.4). In samples collected across northeastern Minnesota, regional temperature differences had no influence on PSM profiles or phytochemical richness but did induce phytochemical turnover in two important moose foods: paper birch and trembling aspen (Figure 2.5); however, warmer temperatures combined with open canopy resulted in distinct PSM profiles for all species and induced phytochemical turnover in all but beaked hazel. Our results demonstrate that woody plants do alter the chemicals they produce in response to abiotic factors; however, different species respond in different ways. Importantly, it seems that canopy cover can modulate the impact of temperature on PSM production—this could have implications on moose diet given the changing patterns of land cover observed in Activity 1. Because the impact of changing PSM profiles on moose is not known, future research that investigates the chemistry of browsed vs. non-browsed plants in different parts of moose range will be important and will help to explain the differences in diet that we have observed in this project (Outcome 2.2). The results from this research were published in *Frontiers in Plant Science* (Berini et al. 2018, in press).

b) Stable isotopic composition of forage plants

After combining our forage species into preference groups (low, medium, high), we were able to reliably separate them using stable isotope compositions measured as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (i.e., the composition of nitrogen and carbon isotopes; Figure 2.6); all pair-wise comparisons are significantly different for $\delta^{13}\text{C}$ (Table 2.1) and $\delta^{15}\text{N}$ (Table 2.2). Statistically significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between forage preference groups indicate that these groups can be used to reliably estimate diet composition (Outcome 2.2).

To determine how isotopic composition of forage plants changed across the region, we collected data on 10 landscape variables at all biomass and forage collection points (Figure 1.3) using the geographic information system (GIS) software ArcGIS 10.3. The variables we considered in these analyses are mean maximum summer temperature (1981-2010; PRISM Climate Group), elevation (U.S. Geological Survey), aspect, slope, disturbance type, percent canopy cover, canopy height, bedrock geology, and water table depth. To evaluate how stable isotope composition of different forage preference groups vary over the landscape, we created linear mixed-effects models in Program R using the lmer command from the lme4 package. We created a null model for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, with easting and northing as our fixed effects and land cover, disturbance type, and bedrock type as our random effects. The fit of our model characterizing landscape-level variation in $\delta^{13}\text{C}$ was significantly improved by the inclusion of slope, water table depth, elevation, and mean-maximum summer temperature. The fit of our model characterizing landscape variation in $\delta^{15}\text{N}$ was significantly improved by the inclusion of water table depth, slope, and elevation. After fitting these models to our data, we created landscape-level predictions using regression kriging to illustrate how the isotope values vary across northeastern Minnesota (Figure 2.7).

Table 2.1 Results of Tukey’s HSD test for $\delta^{13}\text{C}$ of forage preference groups based on Peek (1976). “Difference” refers to the difference between the observed means, whereas “lower” and “upper” refer to the endpoints of the interval. P-values were adjusted for multiple comparisons.

group comparison	difference	lower	upper	p-value
high-aquatics	-1.643	-2.060	-1.226	< 0.0001
low-aquatics	-2.675	-3.082	-2.268	< 0.0001
mid-aquatics	-2.006	-2.431	-1.580	< 0.0001
low-high	-1.032	-1.212	-0.852	< 0.0001

mid-high	-0.363	-0.581	-0.145	0.0001
mid-low	0.670	0.472	0.867	< 0.0001

Table 2.2 Results of Tukey’s HSD test for $\delta^{15}\text{N}$ of forage preference groups based on Peek (1976). “Difference” refers to the difference between the observed means, whereas “lower” and “upper” refer to the endpoints of the interval. P-values were adjusted for multiple comparisons.

group comparison	difference	lower	upper	p-value
high-aquatics	-2.589	-3.168	-2.011	< 0.0001
low-aquatics	-3.707	-4.272	-3.143	< 0.0001
mid-aquatics	-4.392	-4.982	-3.803	< 0.0001
low-high	-1.118	-1.368	-0.868	< 0.0001
mid-high	-1.803	-2.105	-1.501	< 0.0001
mid-low	-0.685	-0.959	-1.411	< 0.0001

V. Moose diet (Outcome 2.2)

To determine how moose diet changes through the growing season and across moose range, we analyzed the stable isotope data from samples of forage plants and moose hair. The plant samples were collected from throughout northeast Minnesota for five consecutive summers (2012-2016; Outcome 2.1) and the moose hair was collected by the Minnesota Department of Natural Resources at radio collaring events from 2013-2014 and at necropsies. Moose shed their winter coat as new hair growth begins in mid to late May and hair growth ends in late August to early September. Because of this seasonal renewal and growth pattern, stable isotopes in hair reflect that of the forage consumed during the summer period—the most important time of food consumption for moose. Thus, hairs collected from fall through early spring allow us to estimate the diet of individual moose during the previous summer. We segmented each hair sample into early summer (the tips of the hair) and late summer (the base of the hair) segments and used stable isotope data from these different segments to estimate seasonal differences in diet.

To estimate diet, we created Bayesian mixings models in Program R using the package MixSIAR, which allowed us to estimate the diet for each individual. We found that early summer diets in the cold region were dominated by medium preference forage, while diets in the moderate and warm regions were dominated by low preference forage and aquatic forage, respectively (Figure 2.8 a). Late summer diets showed that moose in the cold region still focused more on medium preference forage, while the moderate and warm regions had progressively more aquatic forage in their diet (Figure 2.8 b). To test whether diet reflected habitat use we tested whether proportion of aquatic forage in the diet was influenced by the amount of time a given animal spent in wetland habitat. We found a significant, positive relationship, with animals in the warm region tending to have both higher use of wetland habitats and more aquatic plants in their diet (Figure 2.9). In general, animals in the warm region showed stronger selection for aquatic habitats (i.e., they were using them proportionally more than expected, based on wetland availability in their home ranges; Figure 2.10).

VI. Impacts of moose diet on survival (Outcome 2.3)

To determine if summer diet composition was related to the survival of individual moose, we used logistic regression to examine how the summer diet of animals that lived through the following year (n=124) compared to the diets of animals that died before the next summer (n=34). Because of the small number of mortalities, we could not draw robust conclusions; however, our initial analyses indicate that animals that died tended to eat more low-quality forage early in the summer, but less high-quality and more aquatic forage later in the summer (e.g., Figure 2.11). Although these results are tentative, we feel that they may help drive future research into

whether changes in diet can lead to mortality, or whether those changes are indicative of health complications that cause the animals to die in the following season.

VII. Management recommendations (Outcome 2.4)

Based on the findings of this study, we suggest that wildlife researchers at state, federal, and tribal agencies work with foresters in the public and private sectors to identify large blocks of moose range that can be experimentally manipulated or opportunistically monitored. Specifically, there should be paired blocks (treatment / control) that have similar initial conditions in which moose density will be monitored for 1-2 years prior to treatment. If additional radio collars cannot be added to animals in these blocks, the research area can be restricted to locations with existing data on moose space use; in this case, future moose density estimates would have to be conducted by a combination of aerial surveys, pellet counts, browsing surveys, and possibly camera trap grids.

The main cover types to manipulate in treatment plots would be large deciduous and coniferous forest stands. Managing these stands to increase the conversion to a more heterogeneous mixture of tree species will involve selective cutting and possibly planting of trees. While conversion to a true mixed-wood stand will take decades, opening the canopy of some of these stands should increase the density of high-quality forage species (especially aspen and birch) in the understory. Food quantity and quality should be monitored along with the browsing intensity of these plots. Because some moose habitat plots have already been created by researchers in the state, we suggest that those plots be included as reference plots for this research. Other research plots should be created in areas with higher moose populations (e.g., NE portion of moose range) as well as historically moderate densities (e.g., around Isabella, NW of Ely; Figure 1.9). The goal of this management should be twofold: 1) can manipulations create fine-scale increases in habitat use by moose, and 2) at a broad scale, can these manipulations increase the moose population or make it more resilient to changes in predator densities, deer densities, or climate. Clearly it will be difficult to create such a long-term monitoring plan; however, if the timber harvesting can fit within existing forestry goals, then the monitoring of moose densities and forage could be managed on a marginal budget and also provide training for wildlife and forestry students at MN Universities.

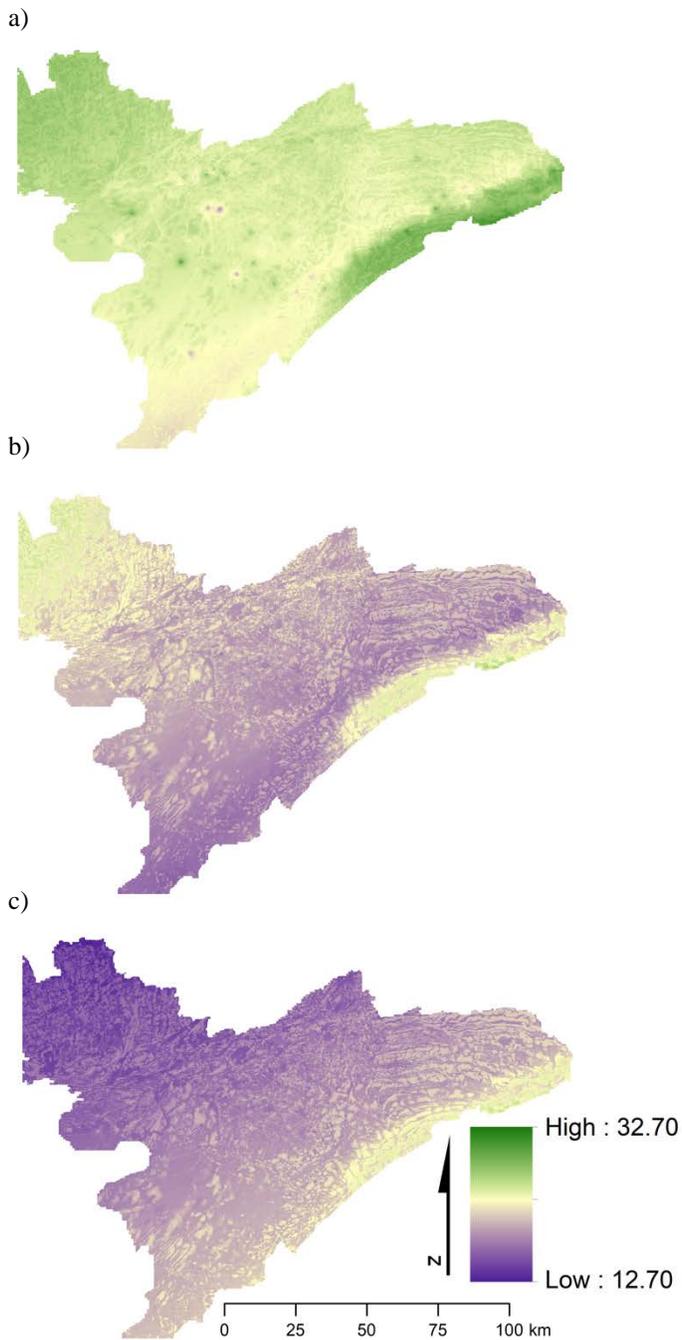


Figure 2.1. Variation in C:N across northeast Minnesota for low (a), medium (b), and high preference (c) forage. Landscape-level predictions were derived via regression kriging using linear mixed effects models. The C:N decrease from low to high preference, indicating an increase in nutrient quality.

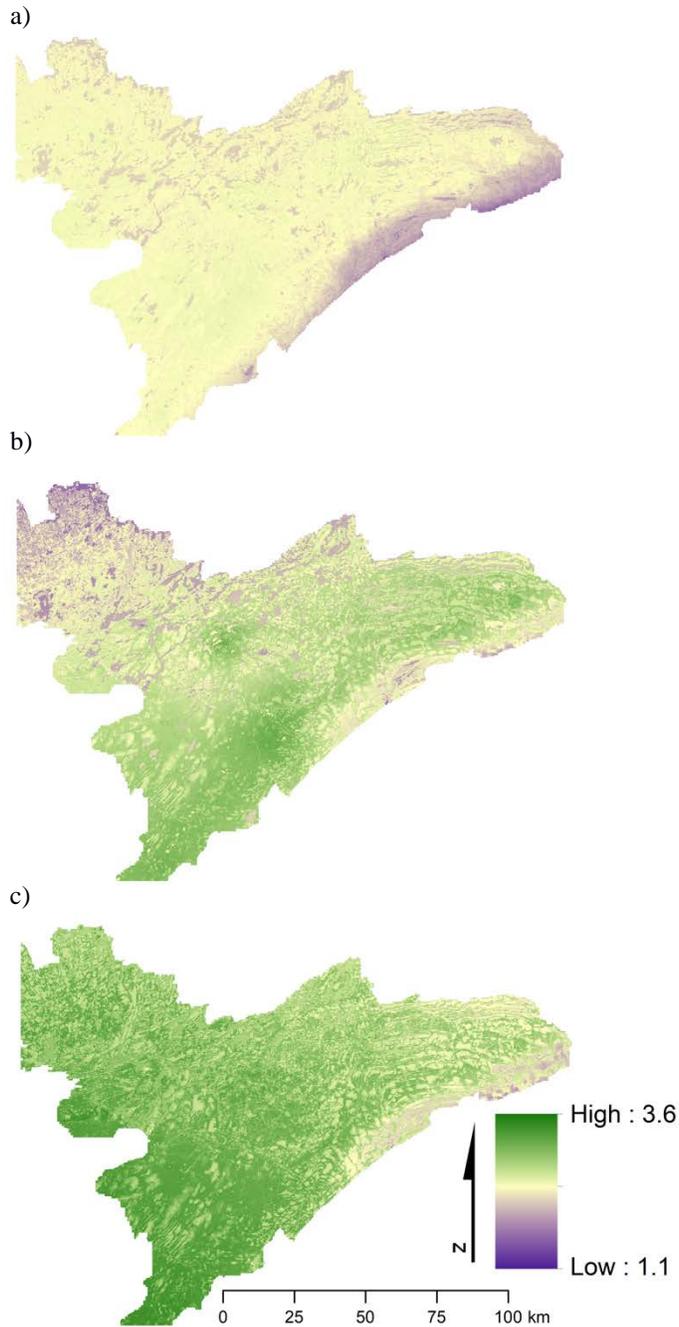


Figure 2.2. Variation in %N across northeast Minnesota for low (a), medium (b), and high preference (c) forage. Landscape-level predictions were derived via regression kriging using linear mixed effects models. The %N values increase from low to high preference, indicating an increase in nutrient quality.

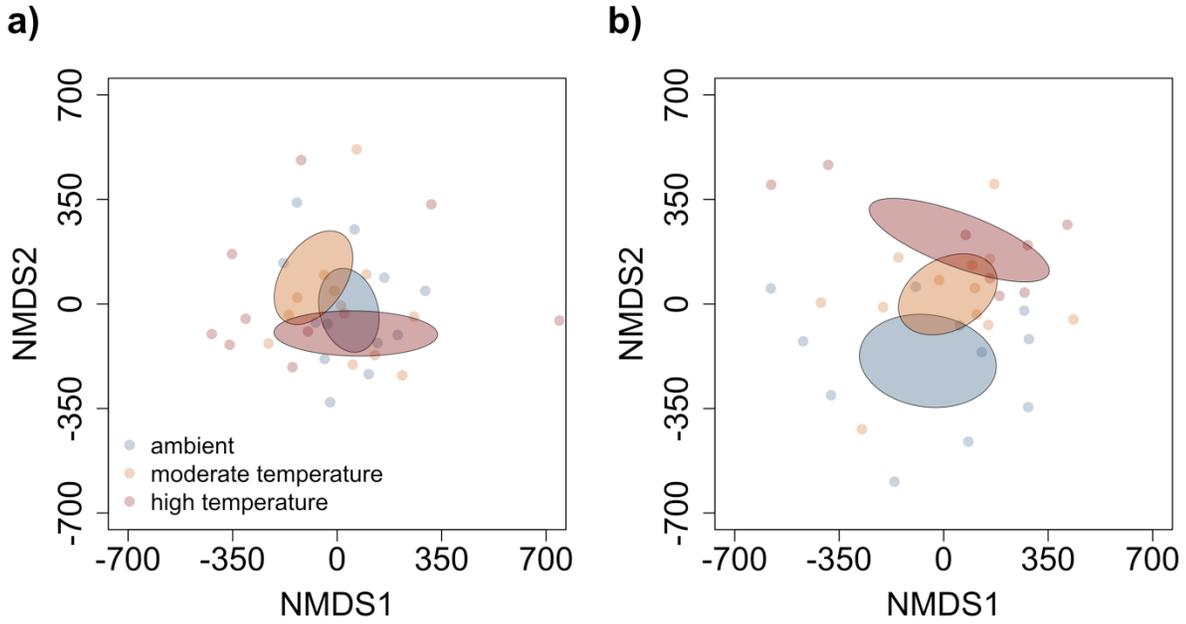


Figure 2.3 Non-Metric Multidimensional Scaling (NMDS) plots detailing the influence of moderate and high-temperature stress on PSM profiles of balsam fir (a) and paper birch (b) in closed overstory. Ellipses represent 95% confidence intervals, based on standard error. In balsam fir (a), both warming treatments exhibit less overlap with each other than with ambient. In paper birch (b), different temperature conditions lead to distinct profiles when compared to each other and ambient.

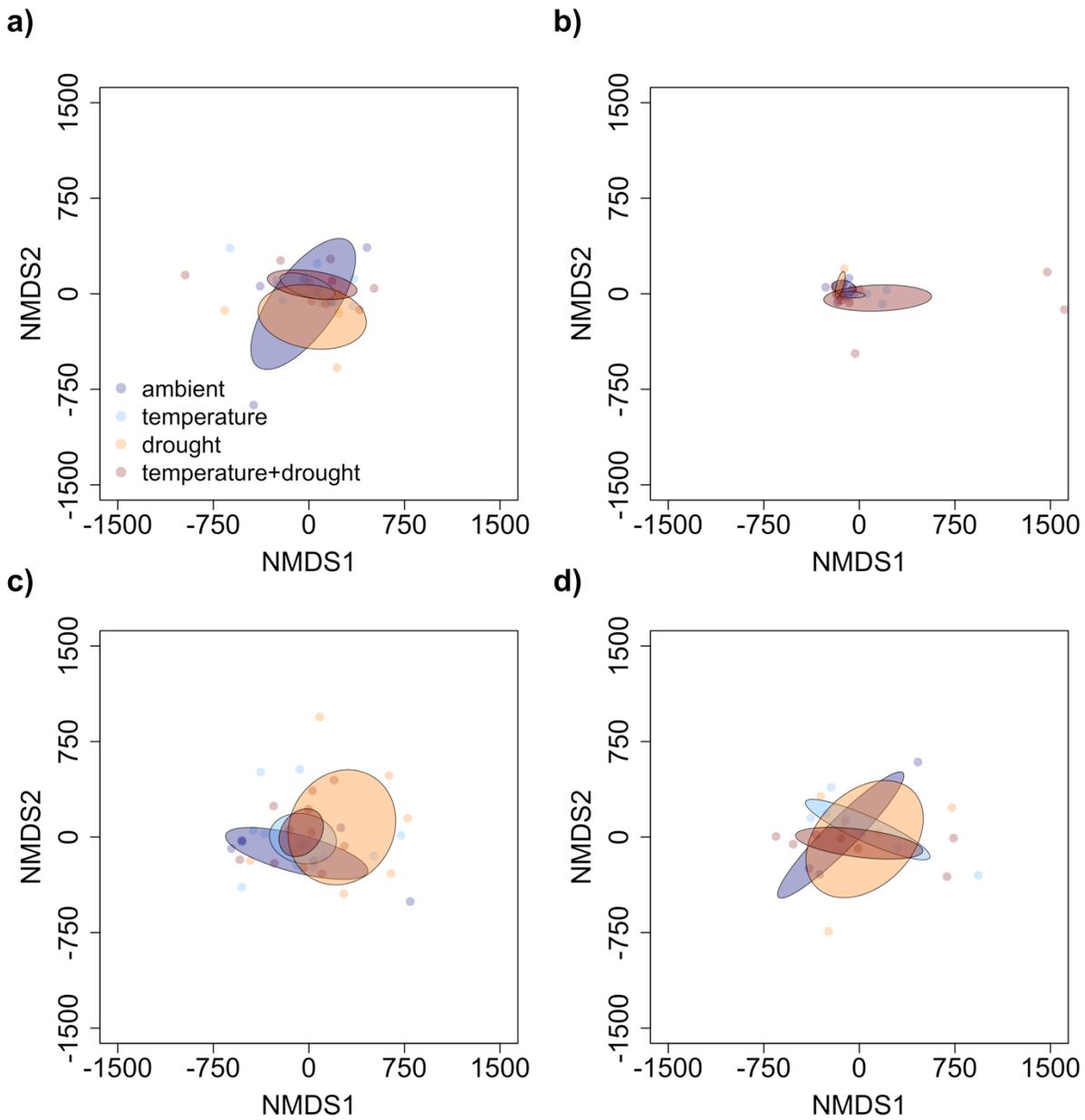


Figure 2.4 Non-Metric Multidimensional Scaling (NMDS) plots detailing the influence of temperature and drought on PSM profiles of balsam fir (a), red maple (b), paper birch (c), and trembling aspen (d) in open overstory. Ellipses represent 95% confidence intervals, based on standard error. There appears to be no discernible pattern between stress conditions and PSM profiles, regardless of species.

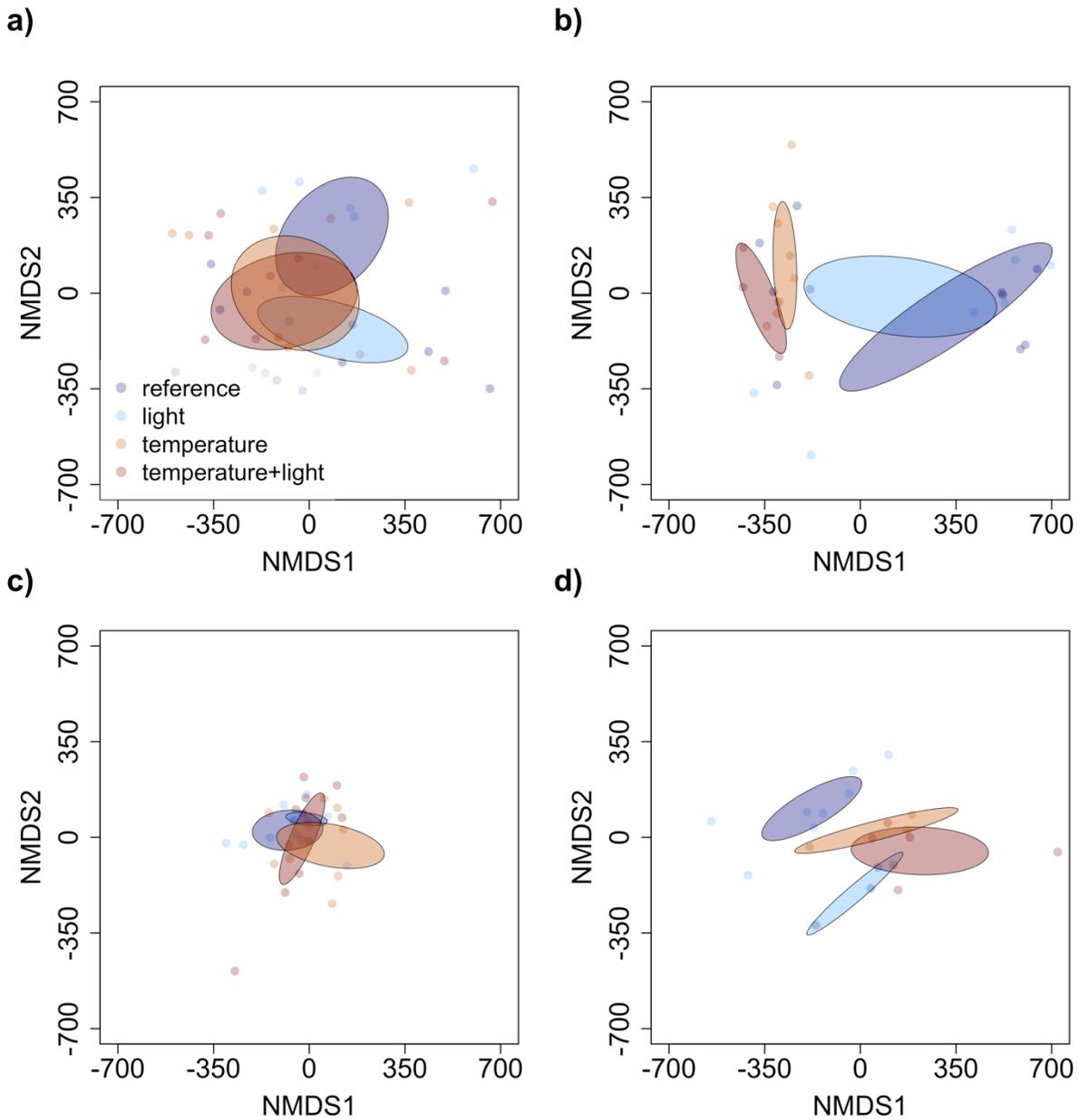


Figure 2.5 Non-Metric Multidimensional Scaling (NMDS) plots detailing the influence of light and temperature stress on PSM profiles of balsam fir (a), paper birch (b), beaked hazel (c), and trembling aspen (d). Ellipses represent 95% confidence intervals, based on standard error. Each species appears to respond to different abiotic conditions in a unique manner. Balsam fir (a) appears to create unique PSM profiles as a function of high light when compared to our reference group (low-light, low-temperature), while paper birch (b) and trembling aspen (d) appear to have distinct PSM profiles for each condition. Beaked hazel (c) exhibits no discernible pattern.

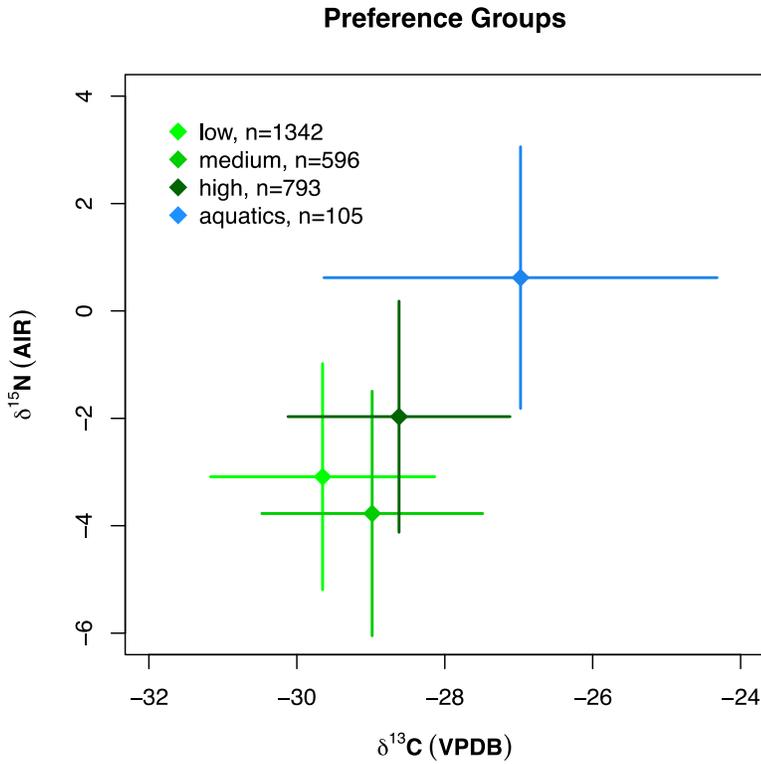


Figure 2.6 Bi-plot representing the mean (points) and standard deviation (lines) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for each forage-preference group. Sample sizes are presented in the legend. Standards used for verifying machine accuracy were air for $\delta^{15}\text{N}$ and Vienne Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$.

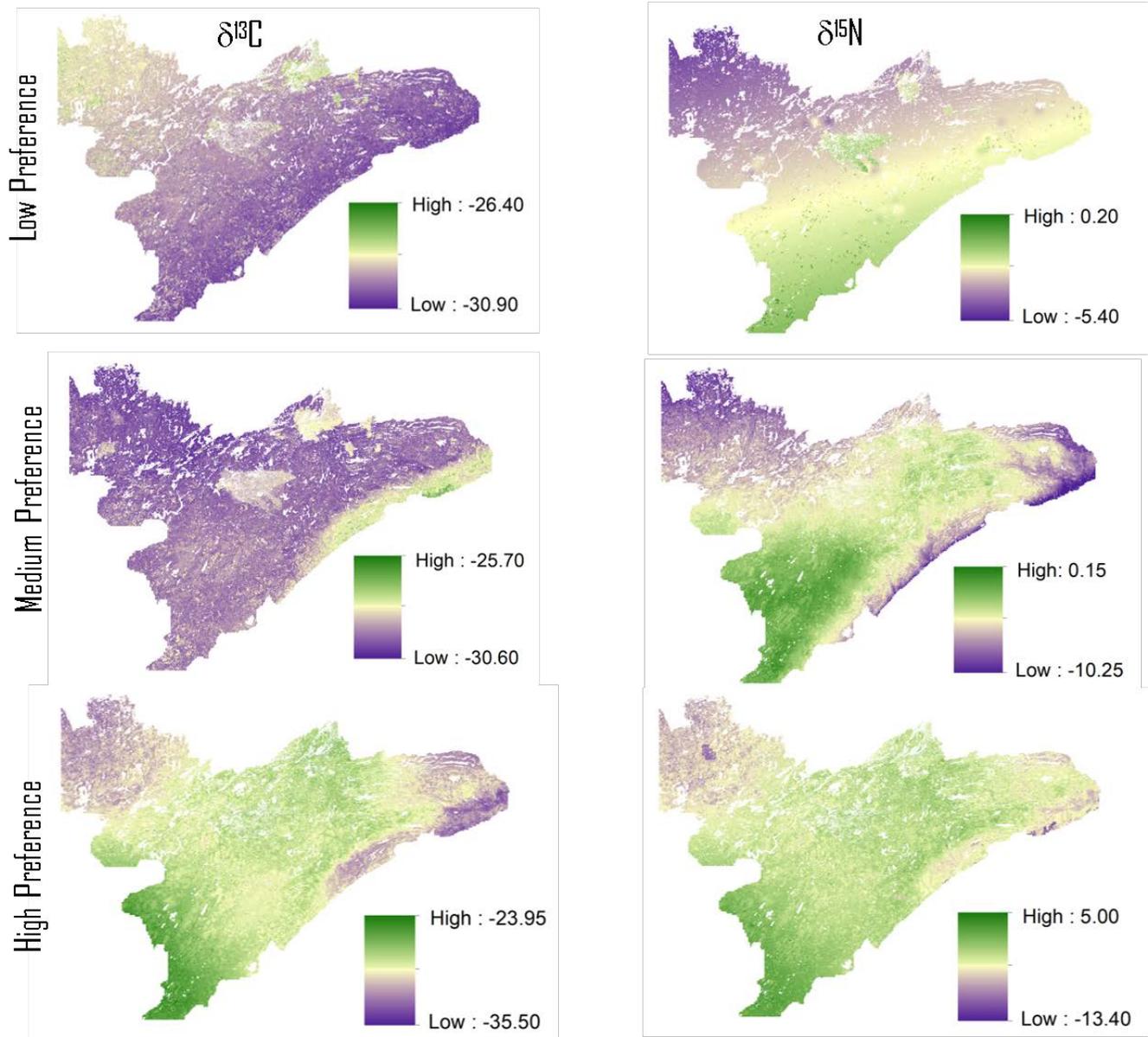


Figure 2.7 Distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across NE Minnesota for all three moose forage preference groups. Landscape-level predictions were derived via regression kriging using linear mixed effects models.

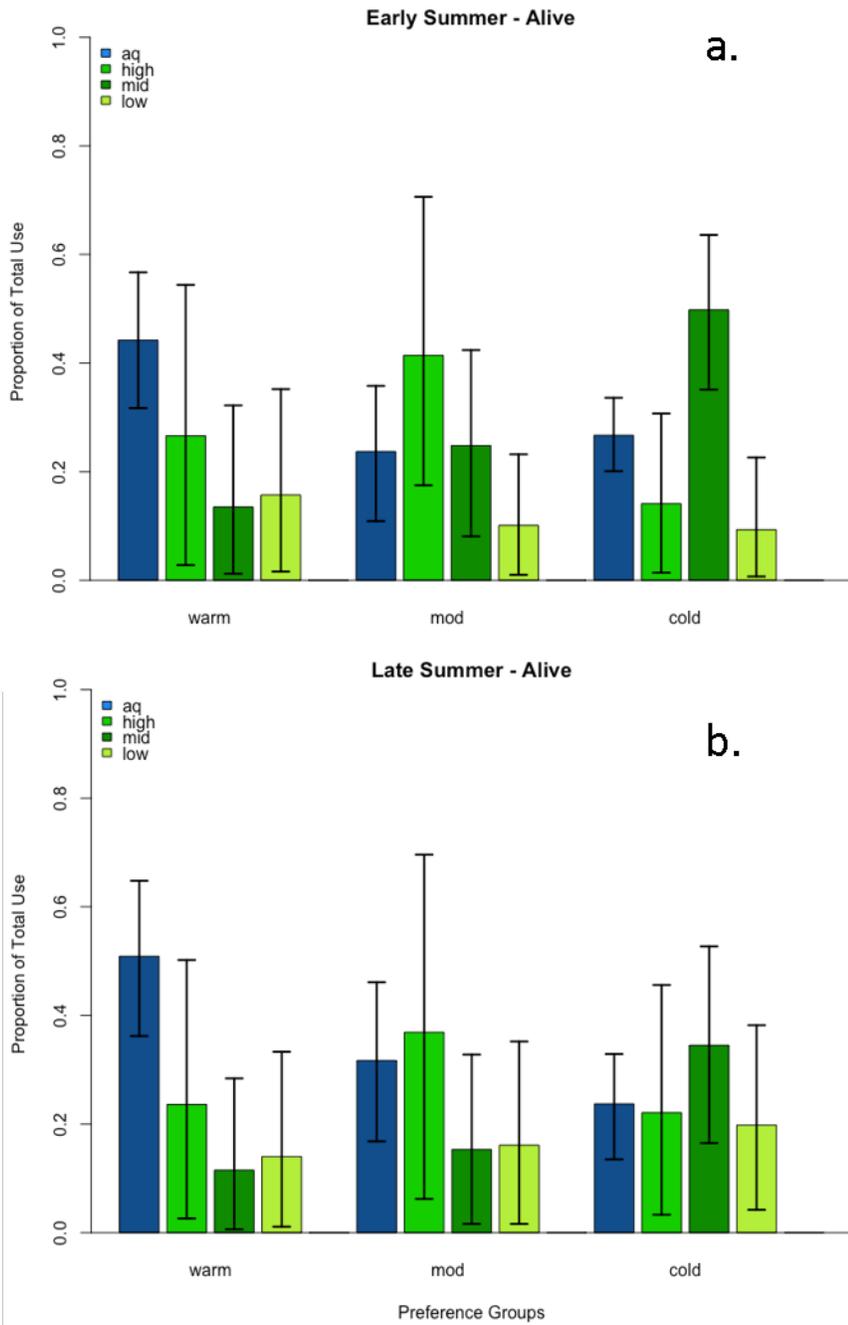


Figure 2.8 Early (a) and late summer (b) diet compositions of moose that survived or died in the following winter. Diets derived from the terminal (early summer) and basal (late summer) portions of hair collected at capture or after mortality.

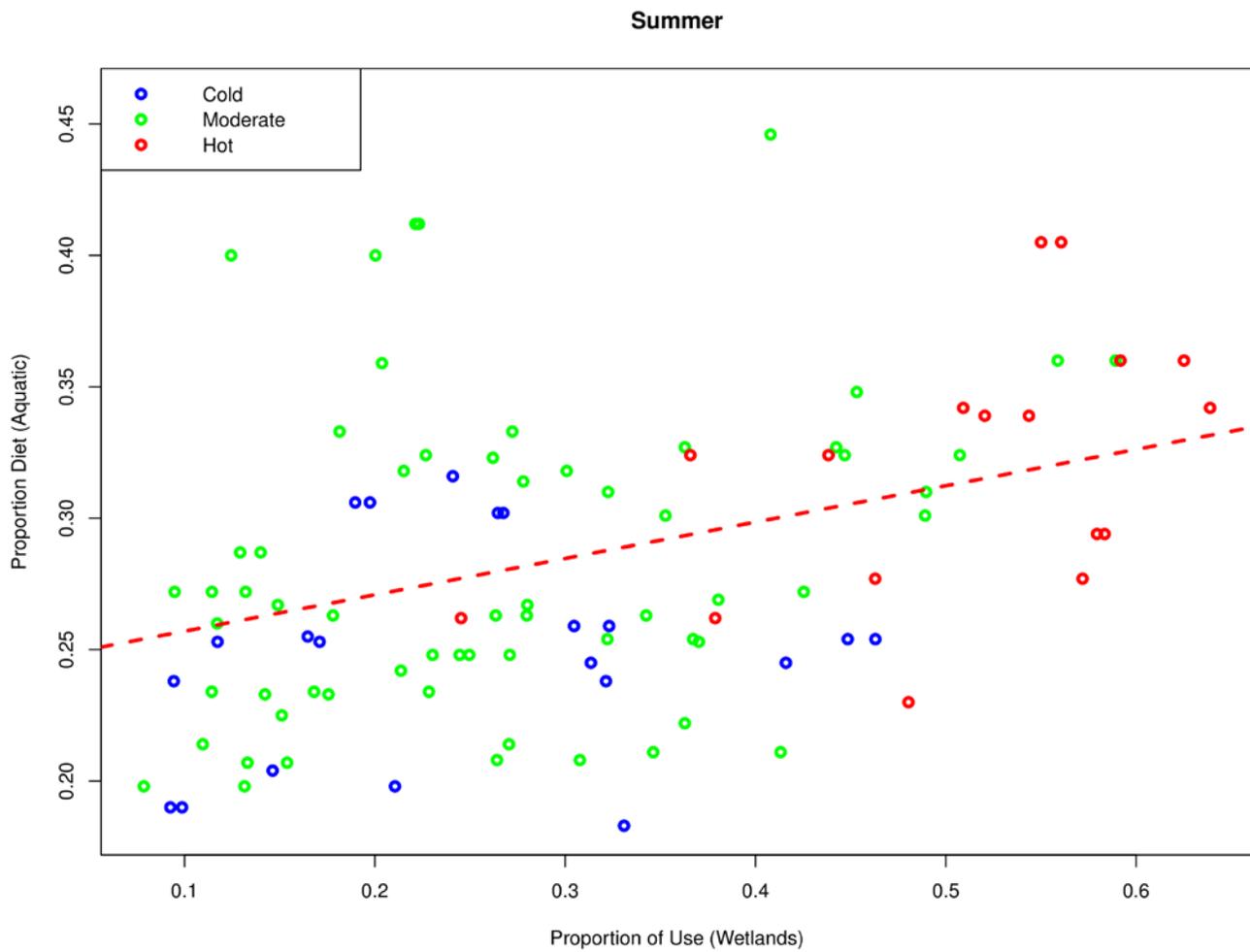


Figure 2.9 Scatterplot and regression of how the summer use of wetlands by moose in each of the three temperature regions is related to the estimated proportion of aquatic for age in the animals' early-summer diet.

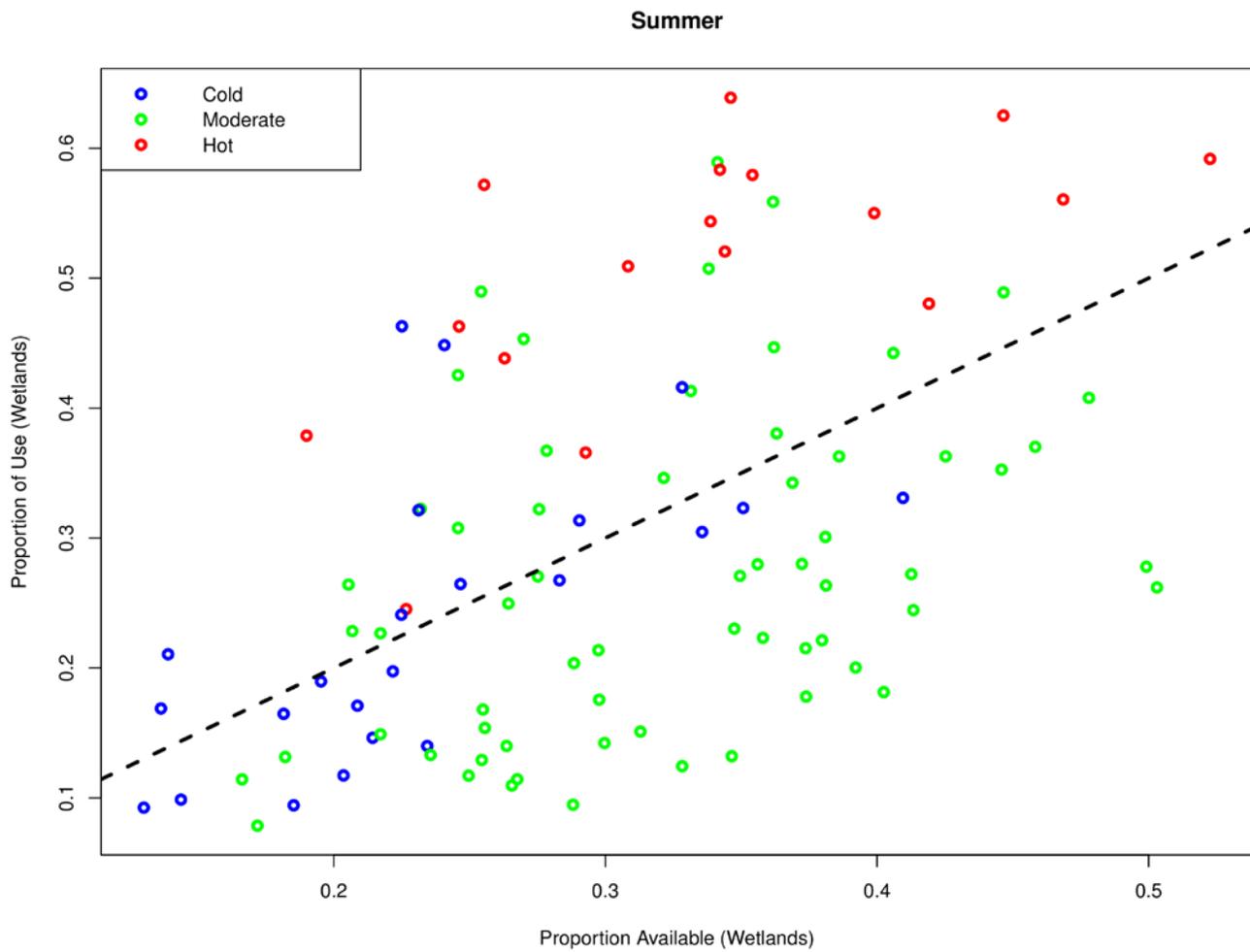
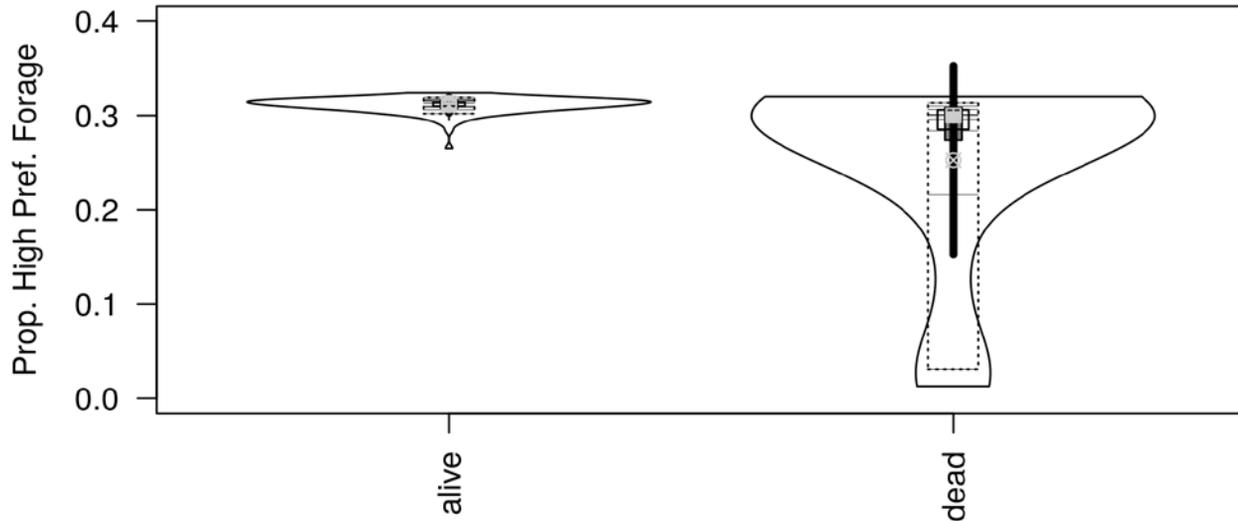


Figure 2.10 Scatterplot of how use of wetlands during summer by moose in the three temperature regions is related to the availability of wetlands within their home ranges. Moose in the warm region tended to select for wetlands (i.e., they used them in a higher proportion than they were available). The dotted line is the 1:1 line; points above that line represent animals that are using wetlands in a greater proportion than available.

Figure 2.11. Violin plots of late summer, high-preference food in the diet of moose that lived through the winter (n=124) compared to those that died (n=34). The dead animals tended have less high-preference forage in their late-summer diets. More research is needed because of the small sample size of dead animals.



V. DISSEMINATION:

Description: A fact sheet that summarizes our findings will be distributed to LCCMR members and land managers at the state and federal level; this will also be made available on the UMN Department of Fisheries, Wildlife, and Conservation Biology website. In addition, several manuscripts will be written and submitted for publication in peer-reviewed journals. Results will be presented at state and national wildlife and ecology conferences (e.g., the annual Minnesota Moose Meeting, The Wildlife Society [both state and national conferences], the Ecological Society of America, and the International Association of Landscape Ecology). All publications resulting from this project will be made available through the FWCB website or Open Access journal websites.

We also expect that there will be a large amount of informal dissemination because we will be working closely with researchers and managers from the Department of Natural Resources, The Nature Conservancy, the Grand Portage Band of Lake Superior Chippewa, the National Park Service, and the US Forest Service. These researchers will take the results of our study into consideration as they make management decisions and will work with us to ensure that our data products and research papers reach a broad audience within their agencies.

Finally, we will continue to pursue public outreach through the Bell Museum of Natural History at UM, which brings University research to the public onsite within the BMNH and offsite through community venues, traveling exhibits, and film productions. We will continue to collaborate with them to develop a unique learning environment that integrates interactive media that presents our on-going research with the existing detail-rich and aesthetically compelling traditional diorama in the BMNH. The decline of moose in Minnesota is of significant public interest, and we expect the presentation of this research to improve public understanding of both the scientific process and the state of this iconic species.

Status as of 2 December 2014:

Forester has given two seminars on the moose population analysis (one to students visiting from Norway, and another to prospective UMN students). Another public seminar is planned for mid December. Forester also gave an extended interview about the moose population to the "Access Minnesota" radio show produced by the Minnesota Broadcasters Association (mid July air date).

Status as of 31 May 2015:

Forester presented at the Annual North American Moose Conference in Granby, CO and gave one public seminar in the Conservation Biology seminar series at the University of Minnesota. Graduate student John Berini presented at a UMN research symposium. Forester continues to work closely with researchers from the MNDNR and Grand Portage Band. Three manuscripts are in the initial stages of drafting. Forester worked with a UROP student at UMN to examine the spatial variation of stable isotope values in paper birch (an important moose food species).

Status as of 31 January 2016:

Berini, Street, and Forester all presented at the Annual Conference of The Wildlife Society in Winnipeg, MB Canada. Forester also gave an invited seminar to the American Association of University Women in Minneapolis, MN. One manuscript is in press, another was submitted for publication and is currently under revision, and a third is in the final stages of drafting.

Street, G. M., J. Fieberg, A. R. Rodgers, M. Carstensen, R. Moen, S. A. Moore, S. K. Windels, and J. D. Forester. 2016. Habitat functional response mitigates reduced foraging opportunity: implications for animal fitness and space use. *Landscape Ecology* – In Press.

Status as of 31 May 2016:

Forester presented the preliminary results from this research at "A Sip of Science" in Minneapolis. A UMN RAP student we work with presented the results of her study (how moose diet changes across NE MN) at the 2016 UMN Undergraduate Research Symposium. John Berini gave a guest lecture on this material for the Principles of Conservation Biology class at UMN and also presented at the Conservation Biology Research Spotlight. We plan to resubmit our manuscript on secondary compounds in moose forage species within the next few weeks and will submit a manuscript on spatially-explicit changes to the moose population by mid summer.

Status as of 1 May 2017:

Forester presented results to a visiting group of students and scholars from Norway in September 2016. He also presented at the International Association of Landscape Ecology conference in Baltimore, MD (April 2017) and will be presenting at the International Congress for Conservation Biology in Cartagena, Colombia in July 2017. Two manuscripts are in revision for submission to journals.

Final Report Summary:

Forester contributed to a multimedia display associated with the moose diorama in the Bell Museum (<https://z.umn.edu/BellMoose>); an excerpt of this interview was featured in the recent PBS special, "Windows to Nature" (<https://z.umn.edu/Windows2Nature>). Forester also gave an extensive interview on the moose population for "Access Minnesota" (<https://z.umn.edu/mooseradio>), presented seven invited talks (UMN Conservation Biology Seminar Series, 2014 & 2017; Minnesota Moose Symposium, 2015; American Association

of University of Minnesota Women, 2015; A Sip of Science, 2016; Boise State University, 2017; Universidade Federal de Mato Grosso do Sul, Brazil 2018), contributed talks to five national or international scientific conferences (North American Moose Conference, Colorado 2015; The Wildlife Society, Winnepeg 2015; US-IALE, Baltimore 2017 & Chicago 2018; International Convention for Conservation Biology, Colombia 2017). John Berini (Ph.D. student) contributed talks to one national (American Society of Mammalogists, Minneapolis 2016) and one international scientific conference (The Wildlife Society, Winnepeg 2015), as well as at two UMN research symposia. Both Forester and Berini included aspects of this research into teaching materials that were delivered to undergraduate students in Wildlife and Conservation Biology courses. Garrett Street also presented at one conference (TWS Winnepeg 2015). Throughout the course of this project, we provided mentorship and training in field, laboratory, and quantitative methods to 12 undergraduate students, five graduate students, and three postdoctoral scholars. Three manuscripts have been published as part of this project:

Street, G. M., J. Fieberg, A. R. Rodgers, M. Carstensen, R. Moen, S. A. Moore, S. K. Windels, and J. D. Forester. 2016. Habitat functional response mitigates reduced foraging opportunity: implications for animal fitness and space use. *Landscape Ecology* 31:1939-1953 doi:10.1007/s10980-016-0372-z.

Fieberg, J. R., J. D. Forester, G. M. Street, D. H. Johnson, A. A. ArchMiller, and J. Matthiopoulos. 2017. Used-habitat calibration plots: A new procedure for validating species distribution, resource selection, and step-selection models. *Ecography* (in press) doi:10.1111/ecog.03123.

Berini, J. L., S. Brockman, A. Hegeman, R. Muthukrishnan, P. B. Reich, R. Montgomery, J. D. Forester. Combinations of abiotic factors differentially alter production of PSMs in woody plants along the boreal-temperate ecotone. *Frontiers in Plant Science* (in press).

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Explanation
Personnel:	\$ 150,969	1 project manager at 8%FTE for 3y; 1 field manager at 38% FTE for 3y; 1 lab manager at 4% FTE for 3 y; 1 lab technician at 8% FTE for 3 y; 1 research associate at 6% FTE for 1 y; 2 undergraduate research assistants at 19%FTE for 3y; 1 PhD student at 14% FTE for 3y.
Professional/Technical/Service Contracts:	\$ 83,944	1 contract for laboratory analysis of plant and tissue samples; 2 contracts for satellite imagery analysis.
Equipment/Tools/Supplies:	\$ 9,980	Lab supplies for stable isotope analysis; field equipment (tapes, sample bags, etc)
Capital Expenditures over \$5,000:	\$ 5,845	High precision GPS for relocating sites and individual plants for resampling.
Fee Title Acquisition:	\$ 0	
Easement Acquisition:	\$ 0	
Easement – Long-term Monitoring, Management, and Enforcement	\$ 0	
Professional Services for Fee Title and	\$ 0	

Easement Acquisition:		
Printing:	\$ 0	
Travel Expenses in MN:	\$ 49,262	Travel to study area by staff and technicians (1 fleet truck for 4mo/y over 3y); lodging and meals for 2-6 crew members for 4mo/y over 3y.
Other:	\$	
TOTAL ENRTF BUDGET:		\$ 300,000

Explanation of Use of Classified Staff:

Explanation of Capital Expenditures Greater Than \$5,000: One Trimble GeoExplorerXT will be purchased for high-resolution field sampling and ground-truthing of satellite classifications. The instrument will continue to be used for similar projects and purposes by the Forester Lab at UMN for the life of the instrument. If the instrument is sold prior to its useful life, proceeds from the sale will be paid back to the Environment and Natural Resources Trust Fund.

Number of Full-time Equivalent (FTE) Directly Funded with this ENRTF Appropriation: 2.98 FTEs

Number of Full-time Equivalent (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0.5 FTEs

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
	\$ 0	\$ 0	
State			
Purchase and maintenance of 15 moose GPS collars (Forester startup)	\$89,463	\$ 50,000	Data from these collars will provide the critical data for this project. We will be able to link where animals spend their time to what they are eating and subsequently their body condition.
Graduate Lab Manager (Fox Stable Isotope Lab, 1mo summer salary + 23.1% health and FICA)	\$2,400	\$ 0	This lab manager will help with the analysis of our samples
Computer equipment dedicated to data analysis and simulation for this project (Forester startup)	\$5,558	\$ 5,558	These computers will provide the computational power to fit the statistical models we will develop in this project.
Foregone ICR funding (52% MTDC, excluding graduate fringe)	\$153,770	\$ 0	
In-kind Services During Project Period: Salaries for Forester (1% match), D'Amato (1% match)	\$6,550	\$ 0	The PIs will be spending substantial time organizing the crews, analyzing data and writing up manuscripts and reports.
TOTAL OTHER FUNDS:	\$ 257,741	\$ 50,000	

VII. PROJECT STRATEGY:

A. Project Partners:

The research team will be led by scientists at the University of Minnesota Departments of Fisheries, Wildlife and Conservation Biology (Dr. James Forester), Earth Sciences (Dr. David Fox), and Forest Resources (Dr. Anthony D’Amato).

Partners include the UMN (Dr. Alan Ek), MNDNR (Dr. Michelle Carstensen, Dr. Glenn DelGiudice), TNC (Mark White), and the Grand Portage Band of Lake Superior Chippewa (Dr. Seth Moore).

B. Project Impact and Long-term Strategy:

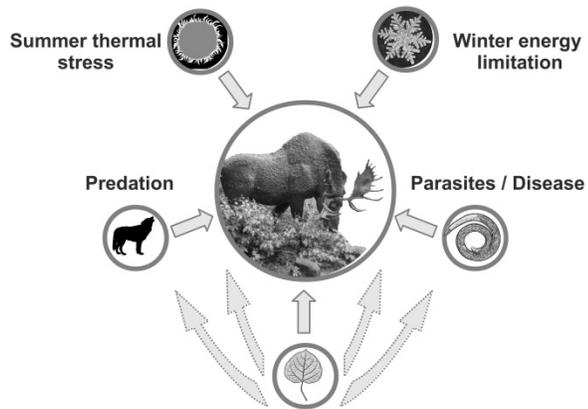
Opportunities to gain insight into the spatial structure of population demographic rates are rare. The proposed work builds on moose research by the MNDNR to examine how this species (of local economic and cultural importance) is responding to changing landscapes. This study will directly address questions of management concern and will also advance managers’ understanding of (1) how animals behaviorally mitigate environmental stress; (2) how behavior and landscape context affect diet, survival, and fecundity; and (3) how broad-scale landscape structure can affect the space use and demographic rates of the moose population. Our ongoing collaborations with state, tribal, and federal agencies will ensure that the research results are broadly disseminated. Likewise, our interaction with the Bell Museum will expose the public to our ongoing efforts to manage and conserve moose in Minnesota.

C. Spending History:

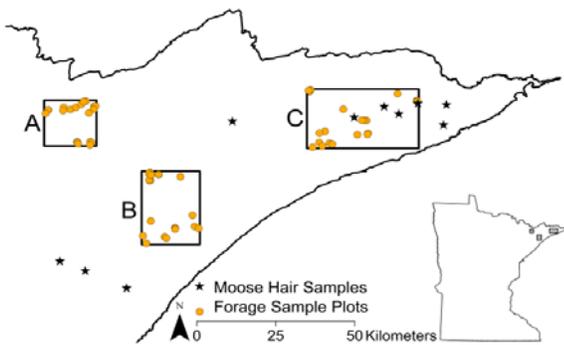
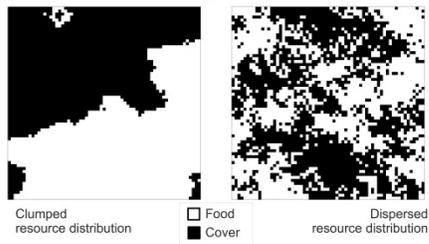
Funding Source	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or FY12-13	M.L. 2013 or FY14
Forester startup funds			52,500	3,058	

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S):



The *distribution* of high-quality food and cover can critically affect animals' susceptibility to predation, parasites, and thermal stress.



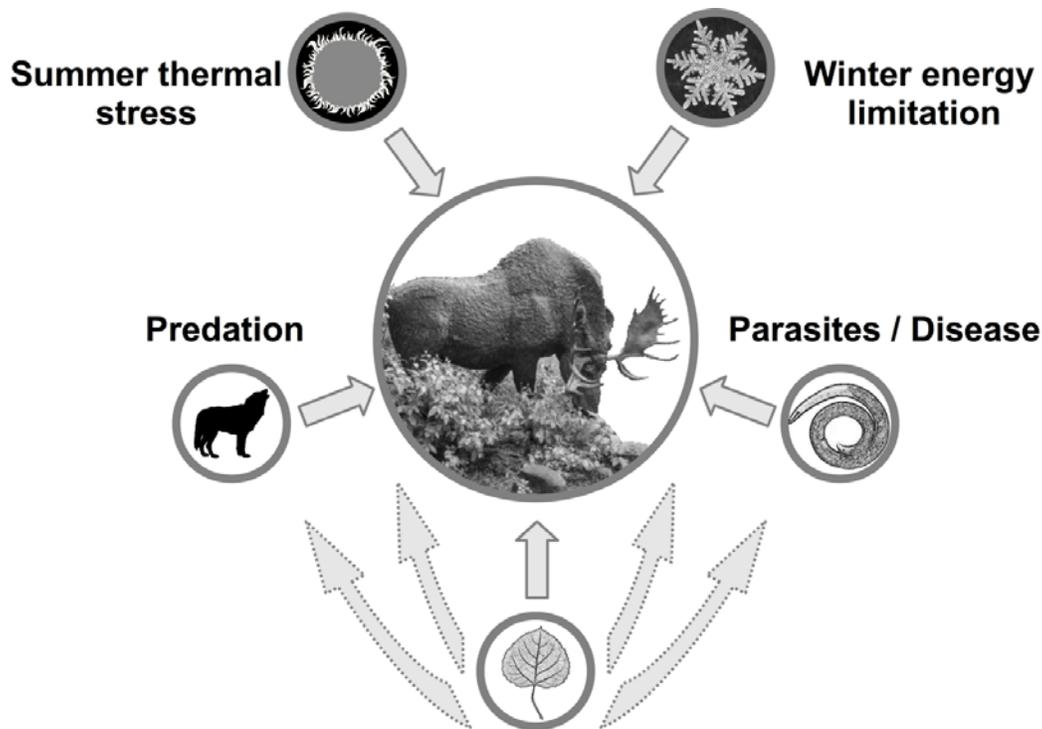
X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM:

See attached Research Addendum

XII. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than 2 December 2014, 31 May 2015, 31 January 2016, 31 May 2016, 31 January 2017, and 30 June 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.



The *distribution* of high-quality food and cover can critically affect animals' susceptibility to predation, parasites, and thermal stress.

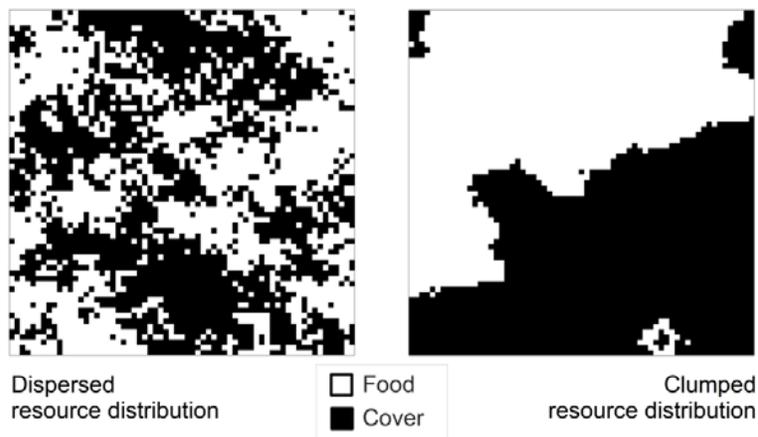


Figure 1. Moose can suffer in the summer heat, run out of food in the winter, fall prey to wolves, or succumb to parasites or disease. The distribution of high-quality food and cover can affect how susceptible animals are to these threats. **We found that: 1) the landscape composition of moose range has changed over 18 years, with mature coniferous forest becoming more dominant, 2) local moose populations had higher growth rates in cooler areas that had large amounts of mixedwood and young forests, and 3) although diets of moose varied across the range, animals that died tended to have eaten less high-quality forage in the previous summer. We suggest that these results be experimentally tested by observing moose behavior and population dynamics in large-scale forest manipulations where the amount of mixedwood and young aspen/birch stands are controlled and the quality and composition of forage species in the understory is monitored.**

Environment and Natural Resources Trust Fund

M.L. 2014 Project Budget



Project Title: Impacts of forest quality on declining Minnesota moose.

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 051 and **Date of Work Plan Approval:** June 4, 2014

Project Manager: James Forester

Organization: University of Minnesota

M.L. 2014 ENRTF Appropriation: \$ 300,000

Project Length and Completion Date: 3 years, 30 June 2017

Date of Report: 2018-08-31

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 5/01/2017	Amount Spent	Activity 1 Balance	Revised Activity 2 Budget 5/01/2017	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
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BUDGET ITEM	Revised Activity 1 Budget 5/01/2017	Amount Spent	Activity 1 Balance	Revised Activity 2 Budget 5/01/2017	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
<i>Linking moose abundance to broad-scale distributions of food and cover that change across space and through time.</i>				<i>Linking the distribution and quality of food and cover to moose diet, body condition and mortality risk.</i>				
Personnel (Wages and Benefits)	\$134,056	\$111,473	\$22,583	\$89,728	\$89,728	\$0	\$223,784	\$22,583
Field manager - \$23,242 (79% salary, 21% benefits); 38% FTE for two years; will lead vegetation sampling effort.								
Faculty (Forester) - \$40,287 (66.2% salary, 33.8% fringe); 8% FTE for four years; will manage project, and lead analysis of moose movement data.								
Faculty (Fox) - \$15,664 (84% salary, 16% benefits); 4% FTE for three years; will supervise the stable isotope analyses								
Lab technician - \$13,076 (73% salary, 27% benefits); 8% FTE for three years; will maintain stable isotope lab equipment and assist with analyses.								
Research Associate (David Wilson) - \$3,769 (73% salary, 27% benefits); 6% FTE for one year; will take lead on collecting and analyzing the FIA data for the moose range.								
Undergraduate research assistants - \$24,340 (100% salary); 2 x 19% FTE over 3 yr; will aid graduate student, field manager, and lab technician with data collection and entry.								
Postdoctoral scholar (Garrett Street) 31,231 (81% salary, 19% fringe) 100% FTE over second 6 months, will compile moose movement data and begin initial habitat-use analysis.								
Postdoctoral scholar (Althea ArchMiller) 18,721(81% salary, 19% fringe) 100% FTE over last 3 months, will analyze habitat data and develop population model								
GIS Technicians (in Falkowski lab, UMN Forest Resources, \$15,00) will classify historic and current satellite imagery.								
PhD student (John Berini) \$19,124 (86% salary, 14% benefits); 14% FTE over first year, 50% FTE over last six months; will collect plants for stable isotope analysis within animal home ranges, will collect moose browse, hair, and fecal pellets during winter, and will take lead on the analysis of moose isotope concentrations.								
PhD student (Andrew Herberg) \$2,340.18 (49% salary, 51% tuition and benefits); 50% FTE for one month; will analyze activity data of moose to predict how foraging behavior changes in different landscapes.								
MS student (Amrit Shandilya) \$16,989 (49% salary, 51% tuition and benefits); 50% FTE last six months; will develop computer program to predict locations of moose under changing landscapes.								
Professional/Technical/Service Contracts								
Isotope analysis (University of Minnesota Stable Isotope Lab) - \$8,963; 956 samples of moose and plant tissue at \$9/sample				\$8,963	\$8,963	\$0	\$8,963	\$0
Isotope analysis (Santa Cruz Stable Isotope Lab) - \$27,894; 2376 samples of plant tissue at \$11.74/sample				\$27,894	\$27,894	\$0	\$27,894	\$0
Chemical composition analysis of plant samples (UMN Metabolomics Lab \$15,000)				\$15,000	\$15,000	\$0	\$15,000	\$0
Development of a 2014 moose-specific habitat classification by combining LiDAR and LANDSAT data (Knight lab \$5000)	\$5,000	\$5,000	\$0		\$0	\$0	\$5,000	\$0
Equipment/Tools/Supplies								
Lab supplies (reagents, weigh tins, gas canisters, and other consumable supplies used for stable isotope analysis) - \$2,769				\$2,769	\$2,769	\$0	\$2,769	\$0
field equipment (measuring tapes, compasses, flagging tape, sample bags, stakes, etc) - \$980	\$600	\$600	\$0	\$380	\$380	\$0	\$980	\$0
Map-grade GPS unit for precise location of field samples and accurate ground truthing of satellite imagery \$5,845				\$5,845	\$5,845	\$0	\$5,845	\$0
Travel expenses in Minnesota								
Travel to study area by project management staff and technicians 4 months/yr for 3 years (1 fleet truck @ \$779/month, \$0.37/mi, 7000 miles/ yr) - \$17,040	\$255	\$255	\$0	\$6,923	\$6,923	\$0	\$7,178	\$0
Room and board for field crew (3 yr of summer and winter field sessions, 4 months/yr, 2-6 crew members at a time, lodging @ \$1,500/mo, meals @ \$1,185/mo) - \$32,222	\$1,000	\$1,000	\$0	\$1,584	\$1,584	\$0	\$2,584	\$0
COLUMN TOTAL	\$140,911	\$118,328	\$22,583	\$159,086	\$159,086	\$0	\$299,997	\$22,583

ECOGRAPHY

Research

Used-habitat calibration plots: a new procedure for validating species distribution, resource selection, and step-selection models

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‘Species distribution modeling’ was recently ranked as one of the top five ‘research fronts’ in ecology and the environmental sciences by ISI’s Essential Science Indicators, reflecting the importance of predicting how species distributions will respond to anthropogenic change. Unfortunately, species distribution models (SDMs) often perform poorly when applied to novel environments. Compounding on this problem is the shortage of methods for evaluating SDMs (hence, we may be getting our predictions wrong and not even know it). Traditional methods for validating SDMs quantify a model’s ability to classify locations as used or unused. Instead, we propose to focus on how well SDMs can predict the characteristics of used locations. This subtle shift in viewpoint leads to a more natural and informative evaluation and validation of models across the entire spectrum of SDMs. Through a series of examples, we show how simple graphical methods can help with three fundamental challenges of habitat modeling: identifying missing covariates, non-linearity, and multicollinearity. Identifying habitat characteristics that are not well-predicted by the model can provide insights into variables affecting the distribution of species, suggest appropriate model modifications, and ultimately improve the reliability and generality of conservation and management recommendations.

Introduction

A variety of data collection and statistical methods are available for linking individuals, populations, and species to the habitats they occupy. Data collection methods range from design-based or opportunistic surveys that result in a set of pooled locations (ignoring any temporal component) (Edwards et al. 2006, Skov et al. 2016) to telemetry studies that result in many locations over time for a small number of individuals (Boyce and McDonald 1999, Pearce and Boyce 2006). A growing number of methods have been proposed for analyzing these different data types, and ‘species distribution



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modeling' (SDM) was recently ranked as one of the top five 'research fronts' in ecology and the environmental sciences by ISI's Essential Science Indicators (Renner and Warton 2013). Regardless of the method used, the underlying objectives are the same: to understand how resources, risks, and environmental conditions influence distribution and abundance patterns (Mayor et al. 2009, Matthiopoulos et al. 2015). A more challenging, but equally important goal is to infer how various perturbations, including climate change and habitat management actions, influence these patterns (Matthiopoulos et al. 2011, Renner and Warton 2013). Unfortunately, SDMs frequently perform poorly when applied to novel environments (Elith et al. 2010, Matthiopoulos et al. 2011, Heikkinen et al. 2012, Wenger and Olden 2012). If we are going to use models to inform decision making, we need to have confidence in their predictions, which in turn requires that we have appropriate methods for model evaluation. Importantly, methods that provide insights into why a model performs poorly (e.g. missing predictors, incorrect functional form, multicollinearity) are more useful than methods that provide only an overall measure of fit.

Much recent literature on model evaluation has focused on the interrelated concepts of model validation, calibration, and discrimination (Pearce and Ferrier 2000, Phillips and Elith 2010, Steyerberg et al. 2010, Harrell 2013, Chivers et al. 2014). Model validation is the process of assessing agreement between observations and fitted or predicted values. When a model (or set of models) is chosen via a data-driven process (e.g. transformations are considered, outliers are inspected and potentially dropped, and multiple models are compared before one or more are selected for inference), evaluations should ideally use out-of-sample data (i.e. data not used to arrive at the model(s); Araújo et al. 2005, Harrell 2013, Muscarella et al. 2014, Naimi and Araújo 2016). The use of out-of-sample data is also critical when evaluating model transferability and is especially challenging if the explanatory variables are correlated among themselves. Prediction error will typically be greater with the new data set unless the correlation among explanatory variables is the same as in the data originally used for model fitting (Dormann et al. 2013). When there is close agreement between observed and fitted/predicted values, we say the model is well calibrated; calibration therefore refers to steps taken to improve agreement between observed and predicted values (e.g. one may choose to 'shrink' regression parameters towards zero to improve out-of-sample predictions when models have been overfit; Harrell 2013, Street et al. 2016). Discrimination, by contrast, describes a model's ability to rank sample units in terms of their likely outcomes (Fielding and Bell 1997, Pearce and Ferrier 2000, Fawcett 2006, Steyerberg et al. 2010).

Calibration and discrimination often go hand-in-hand, though this need not be the case. A model may be well-calibrated but fail to discriminate well if it gives unbiased but highly imprecise estimates. A nice exemplification is given by Ellner et al. (2002), who demonstrated that estimates of extinction probabilities from population dynamic models

are frequently too imprecise to rank individual populations in terms of risk even though they may provide an accurate estimate of the proportion of populations that will cross a quasi-extinction threshold. Conversely, a model may be poorly calibrated, yet have strong discriminating capabilities (Phillips and Elith 2010, Jiménez-Valverde et al. 2013). For instance, population indices may accurately rank sites in terms of their abundance, provided variation in detection probabilities is small relative to variation in abundance, even though indices are biased estimators of population size (Johnson 2008). Researchers routinely use methods such as the area under the receiver operating curve (AUC) to evaluate discrimination of SDMs (Meyer and Thuiller 2006, Heikkinen et al. 2012, Jiménez-Valverde 2012), whereas calibration methods, the focus of this paper, are equally important but underutilized (Phillips and Elith 2010).

We consider methods for validating two general classes of models. The first includes a variety of methods appropriate for survey data pooled over time, in which observed locations are compared to a set of 'background' (or 'control' or 'available') locations generated by randomly or systematically sampling from an area that encompasses the observed locations. Effectively, this approach treats the data as if they were cross-sectional (i.e. the temporal information in the data is ignored when making inferences). Animal telemetry data are also often analyzed in this way, particularly when locations are collected infrequently or if the researcher is interested in habitat use at broad spatial scales (e.g. second or third orders of selection; Johnson 1980). Parallel development of methods for survey data and telemetry data has led to slightly different nomenclatures. The combination of the observed and background points is typically referred to as either presence-background (survey data) or use-availability (telemetry) data and the fitted models as either species distribution models (survey data) or habitat- or resource-selection functions or models (telemetry data). Though a variety of modeling approaches have been used in this context, most – MaxEnt (Elith et al. 2011), spatial logistic regression (Baddeley et al. 2010), weighted distribution theory with an exponential link function (Lele and Keim 2006), and resource utilization distributions (Millspaugh et al. 2006) – can be shown to be equivalent to fitting an inhomogeneous spatial point process model (Warton and Shepherd 2010, Aarts et al. 2012, Fithian and Hastie 2013, Hooten et al. 2013, Renner and Warton 2013).

The second class of models, developed for fine-scale telemetry data, also compares observed locations to a set of background points, but these background points are constrained to areas that are accessible to the animal from the previously observed location (a function of animal movement characteristics and sampling frequency). Each observed location is 'paired' with a set of background/available points, resulting in highly stratified data. These data types are typically analyzed by fitting a conditional logistic regression (or equivalently, a discrete choice) model (Arthur et al. 1996, Manly et al. 2002), and the fitted models are referred to as

step-selection functions (SSF) (Fortin et al. 2005, Forester et al. 2009, Thurfjell et al. 2014) or integrated step-selection functions (Avgar et al. 2016). Although these two classes of models share some features, calibration techniques developed for presence-absence (Harrell 2013) or presence-background data (Boyce et al. 2002, Johnson et al. 2006, Phillips and Elith 2010) do not easily generalize to step-selection functions because the data used to fit the latter models are highly stratified. Further, little work has been done to develop methods for validating step-selection models (but see Street et al. 2016).

The popularity of SDMs, their propensity to fail when used to predict distributions in novel environments, and the current lack of sufficient diagnostics for evaluating models, especially those developed to analyze fine-scale telemetry data, are causes for concern. Here, we introduce a new method for model validation that can be applied across the entire spectrum of SDMs. Rather than focus on validating a binary response variable ($Y=1$ for presence locations and 0 for background locations), we proposed to validate models by comparing distributions of the explanatory variables at the observed and predicted presence locations – i.e. the habitat characteristics associated with the used locations. These plots, which we refer to as used-habitat calibration plots or UHC plots, complement existing approaches for validating traditional (non-stratified) species distribution or habitat selection models and also fill a void by providing a way to validate step-selection functions. Through a series of simulated and empirical examples, we show how UHC plots can help with three fundamental challenges of habitat modeling: identifying missing covariates, non-linearity, and multicollinearity.

Pooled-survey data examples

We begin by considering two simple simulation examples where the variables influencing species distribution patterns are known. These examples are useful for testing if model validation tools return sensible and informative results under known model misspecifications. In particular, we will use these examples to explore the ability of model validation tools to diagnose a missing predictor or the need for a non-linear term. To understand the data-generating process, let $f^a(x)$ describe the available or background distribution of covariate(s) x in environmental space (i.e. $f^a(x)$ gives the relative frequency with which different values or levels of x occur across the entire landscape). Further, let $f^u(x)$ describe the distribution of the covariate(s) at used (i.e. presence) locations.

In our first example, constructed to explore the impact of a missing predictor, the species distribution was driven by elevation (x_1) and precipitation (x_2), with the species preferring sites at higher elevations and with lower levels of precipitation. In this example, the distribution of x_1 and x_2 in environmental space was assumed to be normal and centered to have mean 0: $f^a(x_1, x_2) = N(0, \Sigma)$. We considered

three different data-generating scenarios in which we set $\text{var}(x_1) = \text{var}(x_2) = 4$, but varied $\text{cor}(x_1, x_2) = \rho_{x_1, x_2}$ to explore how the effect of a missing predictor depends on the correlation among predictor variables. In the first scenario, we set $\rho_{x_1, x_2} = 0$ in both training and test data sets. In the second scenario, we set $\rho_{x_1, x_2} = -0.3$ in both training and test data sets, and in the third scenario, we set $\rho_{x_1, x_2} = 0.3$ in the training data set and $\rho_{x_1, x_2} = -0.3$ in the test data set. For each of these three scenarios, we formed training data by choosing 100 presence locations, with the probability of selection proportional to $\exp(0.5x_1 - x_2)$. We combined these locations with a set of 10 000 randomly generated background points from $f^a(x_1, x_2)$. We set $Y=1$ for the 100 presence locations and $Y=0$ for the 10 000 background locations. We used the same approach to form a test data set of the same size (100 presence and 10 000 background locations).

We fit two different logistic regression models to the training data. First, we fit a model that included only elevation. Second, we fit a model that included both elevation and precipitation (the correct model). The estimated regression coefficients for elevation and precipitation were close to the data-generating values of 0.5 and -1 whenever we fit the correct model (i.e. $y \sim \text{elev} + \text{precip}$; Table 1). The coefficient for elevation was also close to the data-generating value of 0.5 if we fit the model without precipitation, provided $\rho_{x_1, x_2} = 0$. By contrast, the coefficient for elevation in the model without precipitation was too high when $\rho_{x_1, x_2} = -0.3$ and too low when $\rho_{x_1, x_2} = 0.3$ (Table 1). This type of bias, referred to as omitted-variable bias, is well-known and is a function of $\text{cor}(x_1, x_2)$ and $\text{cor}(y, x_2|x_1)$ (Clarke 2005).

We considered a second example to explore the effect of model misspecification, where the species distribution exhibits a non-linear response to temperature (x_3). The optimal temperature for this species was set at $x_3 = 1$, with habitat suitability dropping off for warmer and colder temperatures. We again considered centered values of x_3 , assumed to be normally distributed on the landscape with $f^a(x_3) = N(0, 4)$. We formed test and training data using the same approach as in the previous example, but with the probability of selecting locations proportional to $\exp(2x_3 - x_3^2)$.

Table 1. Estimated regression parameters ($\hat{\beta}$) and their standard errors (SE) for logistic regression models fit to training data in the first cross-sectional data simulation. The marginal distribution of elevation (x_1) and precipitation (x_2) on the landscape was given by a multivariate normal distribution with mean vector = (0,0), and $\text{var}(x_1) = \text{var}(x_2) = 4$. We considered three different data-generating scenarios in which we varied $\text{cor}(x_1, x_2)$ (= 0, -0.3, or 0.3). The true species distribution was proportional to $\exp(0.5x_1 - x_2)$.

$\text{cor}(x_1, x_2)$	$Y \sim \text{elev}$		$Y \sim \text{elev} + \text{precip}$			
	$\hat{\beta}_{x_1}$	SE	$\hat{\beta}_{x_1}$	SE	$\hat{\beta}_{x_2}$	SE
0.00	0.42	0.05	0.42	0.06	-1.04	0.07
-0.30	0.80	0.06	0.52	0.06	-0.99	0.07
0.30	0.27	0.05	0.57	0.06	-0.97	0.06

Table 2. Estimated regression parameters ($\hat{\beta}$) and their standard errors (SE) for logistic regression models fit to training data in the second cross-sectional data simulation. The marginal distribution of x_3 on the landscape, $f^a(x_3)$, was Normal: $f^a(x_3) = N(0,4)$. The relative probability of use (or presence) was proportional to $\exp(2x_3 - x_3^2)$.

Model	$\hat{\beta}_{x_3}$	SE	$\hat{\beta}_{x_3^2}$	SE
$y \sim x_3$	0.24	0.05		
$y \sim x_3 + x_3^2$	2.21	0.35	-1.05	0.17

We fit a model with only a linear effect of temperature on the logit scale and another that also included a quadratic term (the correct model). The coefficient for temperature was too low when we fit the model with only temperature, but the coefficients were close to the data-generating values of 2 and -1 when both temperature and temperature² were included in the model (Table 2).

In subsequent sections, we evaluate each model's ability to predict presence locations in the test data. R code (R Core Team) for generating the data and performing all analyses in the paper, along with any associated output, have been archived within the Data Repository for the Univ. of Minnesota: <<http://doi.org/10.13020/D6T590>> (Fieberg et al. 2016). We have also included functions for simulating and analyzing these data in an R package named 'uhcplots' hosted on GitHub (Fieberg and ArchMiller 2016). This package can be downloaded using the `install_github()` function in the devtools library: `devtools::install_github("aaarchmiller/uhcplots")`.

Calibration plots

Methods for validating models include goodness-of-fit tests, diagnostic plots to assess model assumptions (e.g. residual versus fitted plots), and calibration plots of observed versus predicted values, where the latter are formed using cross-validation or bootstrapping (Phillips and Elith 2010, Harrell 2013). Calibration plots are particularly useful since they provide an honest measure of model fit by using different data sets to fit and then evaluate the model. Unfortunately, calibration plots have received relatively little attention in the species distribution literature (but see Phillips and Elith 2010). Because many ecologists are unfamiliar with calibration plots, we will work towards our suggested approach by first detailing the steps necessary for producing a calibration plot when logistic regression is used to model binary (presence-absence) data. We then describe how calibration plots have been modified to work with presence-background data and illustrate these methods in conjunction with the above simulated data examples. With this foundation in place, we develop an alternative method of model calibration that focuses on the distribution of habitat characteristics at locations where the species is present.

Calibration plot for presence-absence data

Let Y represent the presence or absence of a species, a Bernoulli random variable with mean that is dependent on covariates X , $E[Y|X] = P(Y=1|X) = \pi$. Further, let (x^{train}, y^{train}) refer to predictor and response data, respectively, used to fit the model and (x^{test}, y^{test}) refer to predictor and response data used to validate model predictions. In real applications, test and training data may be formed by data splitting, using k -fold cross-validation (Muscarella et al. 2014), or by sampling data with replacement multiple times (i.e. separate bootstrap samples; Harrell 2013, Fieberg and Johnson 2015). Alternatively, the model may be validated with data collected at another point in time or space, leading to a more stringent test of a model's predictive ability. To produce a calibration plot with presence-absence data:

1) Estimate regression parameters, $\hat{\beta}^{train}$, by fitting a logistic regression model to the *training* data (x^{train}, y^{train}) .

2) Form predictions for the *test* data using x^{test} and the parameters estimated from the *training* data (i.e. $\hat{\beta}^{train}$ from

$$\text{step [1]): } \hat{\pi}^{test} = \frac{\exp(x^{test} \hat{\beta}^{train})}{1 + \exp(x^{test} \hat{\beta}^{train})}.$$

3) Form a calibration plot using one of three options. Option 1: bin the y^{test} data (e.g. based on quantiles of $\hat{\pi}^{test}$). Plot the proportion of values where $y^{test} = 1$ in each bin versus mean $\hat{\pi}^{test}$ in each bin. Option 2: fit a new logistic regression model to the test data, considering a single predictor, $x^{test} \hat{\beta}^{train}$ (i.e. the logit of the predicted values): $\text{logit}(E[Y^{test} | X^{test}]) = b_0 + b_1(x^{test} \hat{\beta}^{train})$. Plot the fitted line with confidence intervals. Option 3: fit a more flexible, non-linear model (e.g., using regression or smoothing splines): $\text{logit}(E[Y^{test} | X^{test}]) = f(x^{test} \hat{\beta}^{train})$, and plot the fit of the model with confidence intervals.

If the model is well-calibrated, we should see the binned values (option 1) or the fitted curves (options 2 and 3) line up well with the 1:1 line. Further, estimates of (b_0, b_1) should be close to $(0, 1)$ (option 2) if the model is well-calibrated. If estimates of (b_0, b_1) are far from $(0, 1)$, then one may choose to use (b_0, b_1) to re-calibrate the model (Giudice et al. 2012, Harrell 2013).

Calibration plots for presence-background data

Presence-background data differ from presence-absence data in that the zeros (the background data) may be utilized by the species (i.e. they are not 'true absences'). Boyce et al. (2002) and Johnson et al. (2006) developed a calibration plot for presence-background data that has been widely used to validate habitat selection models fit to telemetry data using logistic regression. Rather than use predicted probabilities from the fitted logistic regression model in step [2], Boyce et al. (2002) suggested using $w(x^{test} \hat{\beta}^{train}) = \exp(x^{test} \hat{\beta}^{train})$ for model calibration. Although this approach might at

first appear to be ad hoc, it can be justified by recognizing that most methods for analyzing presence-background data, including logistic regression, can be shown to be equivalent to fitting an inhomogeneous Poisson process (IPP) model (Warton and Shepherd 2010, Aarts et al. 2012, Fithian and Hastie 2013, Hooten et al. 2013, Renner and Warton 2013). The likelihood for an IPP model, conditional on n_u total used (i.e. presence) locations from area A , is given by:

$$L(y_i | x_i, \beta) = \prod_{i=1}^{n_u} \frac{\exp(x_i \beta)}{\int_A \exp(x(s) \beta) ds} \quad (1)$$

The n_a randomly (or systematically) sampled available (i.e. background) points serve to approximate the integral in the denominator:

$$L(y_i | x_i, \beta) \approx \prod_{i=1}^{n_u} \frac{\exp(x_i \beta)}{\sum_{j=1}^{n_a+n_u} w_j \exp(x_j \beta)} \quad (2)$$

where the w_j are quadrature weights used to approximate the integral in Eq. (1) using numerical integration techniques (ideally, the number of background points should be large enough that regression parameter estimators do not change with the addition of more points; Warton and Shepherd 2010). Thus, conditional on the set of used and available points (n_u, n_a), the probability of selecting each point is proportional to $\exp(x\beta)$.

Boyce et al. (2002) and Johnson et al. (2006) suggested using k -fold cross-validation to form a binned calibration plot. After forming predictions via cross-validation, the plot is constructed via the following steps.

1) Bin the y^{test} data using quantiles of $w(x^{test} \hat{\beta}^{train})$ and calculate the mean value of $w(x^{test} \hat{\beta}^{train})$ in each bin, \bar{w}_i ($i = 1, 2, \dots, n_{bins}$).

2) Determine the number of used locations in each bin, n_u^i .

3) Determine the expected number of used locations in each bin, $E[n_u^i] = n_u^{test} \frac{\bar{w}_i}{\sum_{k=1}^{n_{bins}} \bar{w}_k}$, where n_u^{test} is the total number of used (i.e. presence) locations in the test data set. (Note: this equation can be modified slightly if the number of locations in each bin is not constant, see Johnson et al. 2006.)

4) Plot n_u^i versus $E[n_u^i]$ along with a 1:1 line. As with presence-absence calibration plots, models with adequate fit should result in points that largely follow the 1:1 line.

Boyce et al. (2002) also advocated for calculating the Spearman correlation between n_u^i and $E[n_u^i]$. As noted by Phillips and Elith (2010), the Spearman correlation provides an alternative, non-parametric method for assessing calibration. Johnson et al. (2006) also suggested fitting a linear regression model relating n_u^i to $E[n_u^i]$, which should result in intercept and slope estimates close to 0 and 1, respectively, if the model is well-calibrated. Lastly, we note that Phillips and Elith (2010) proposed a similar presence-background calibration plot using statistical smoothers to evaluate fit, thus avoiding the need to bin the data.

Application of presence-background calibration plots to pooled-survey data examples

Following Johnson et al. (2006), we constructed presence-background calibration plots for the models fit to each of the simulated pooled-survey data sets (Fig. 1, 2). In the first example, both models resulted in calibration plots that roughly followed the 1:1 line as long as ρ_{x_1, x_2} was the same in the test and training data (Fig. 1A–D). When ρ_{x_1, x_2} differed between the test and training data, the calibration plot for the elevation-only model differed significantly from the 1:1 line (Fig. 1E), whereas the correct model remained well-calibrated (Fig. 1F). Another noteworthy feature of the calibration plots, particularly those for the correct model (Fig. 1B, D, F) or the elevation-only model in the case where $\rho_{x_1, x_2} = -0.3$ for training and test data (Fig. 1C), is a clustering of observed and expected counts near 0, except for the largest bin. This tight clustering reflects the high discriminatory ability of the models (i.e. they are able to clearly identify those points that have the highest relative probability of use).

In the second example, the model containing only a linear effect of temperature resulted in a calibration plot with points that were widely scattered, and although the regression line was close to the 1:1 line, the R^2 is 0.04, suggesting the model did a poor job of predicting presence points in the test data (Fig. 2A). By contrast, the points in the calibration plot for the correct model, containing both temperature and temperature², closely followed the 1:1 line ($R^2 = 0.99$; Fig. 2B) suggesting this model was well-calibrated.

In summary, using presence-background calibration plots, we were able to correctly identify poorly calibrated models when we were missing an important predictor (but only when the correlation among predictor variables changed between training and test data sets; Fig. 1E) or when we needed to include a non-linear term (Fig. 2A). By themselves, however, these plots provide little additional insight into what might be causing the lack-of-fit or ways that the model might be improved.

Used-habitat calibration (UHC) plot

A variety of residual plots (e.g. partial residual plots, added variable plots) have been developed to evaluate the potential for missing predictors or the need for non-linear terms in linear and generalized linear models (Kutner et al. 2005, Moya-Laraño and Corcobado 2008). Here, we develop a simple method for producing calibration plots that accomplish these same goals, but we use out-of-sample predictions. Specifically, we develop calibration plots that evaluate how well a model predicts the characteristics associated with the used (presence) locations. We call this type of plot a used-habitat calibration plot (or UHC plot) and describe the steps for producing such plots below (see Fig. 3 for an illustration of the steps in the context of the first simulation example using the model with elevation but without precipitation).

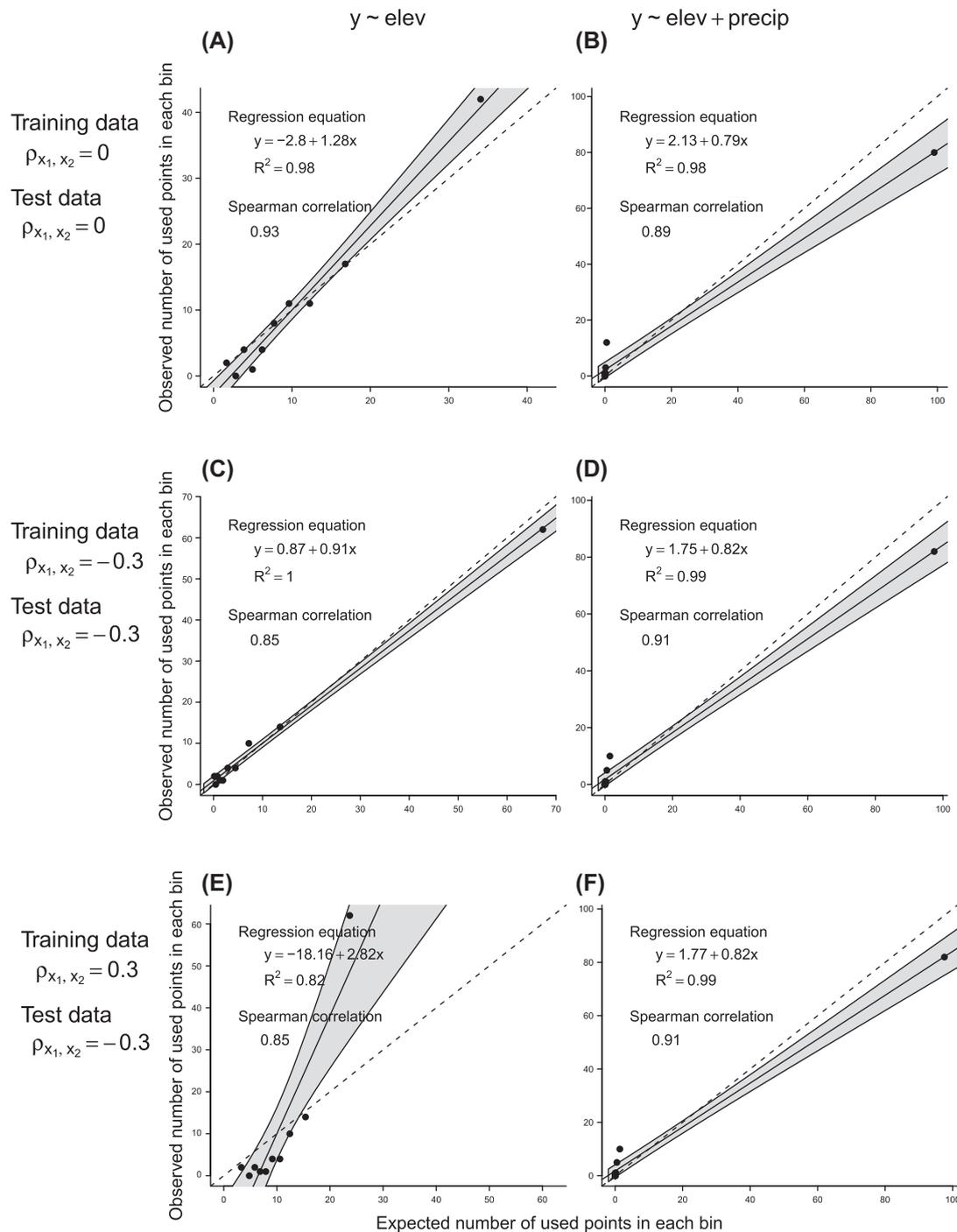


Figure 1. Presence-background binned calibration plots using the method outlined in Johnson et al. (2006) applied to simulated data for a species whose distribution was driven by elevation (x_1) and precipitation (x_2). The marginal distribution of x_1 and x_2 on the landscape, $f^i(x_1, x_2)$, was Normal: $f^i(x_1, x_2) = N(0, \Sigma)$. We considered three different data-generating scenarios in which we set $\text{var}(x_1) = \text{var}(x_2) = 4$, but varied $\text{cor}(x_1, x_2) = \rho_{x_1, x_2}$ (represented by separate rows of panels). The relative probability of use (or presence) was proportional to $\exp(0.5x_1 - x_2)$. Panels depict observed versus expected numbers of presence locations within 10 bins formed using estimated relative probabilities of selection, $w(x^{test} \hat{\beta}^{train}) = \exp(x^{test} \hat{\beta}^{train})$, where x^{test} is a matrix of covariates in the test data set and $\hat{\beta}^{train}$ is a vector of regression parameter estimates obtained by fitting one of two logistic regression models to the training data (the two models are represented by the different columns). Overlaid is a regression line (black line with shaded 95% confidence intervals) relating observed and expected numbers of presence locations in each bin. A well-calibrated model should closely follow the 1:1 line (dashed line).

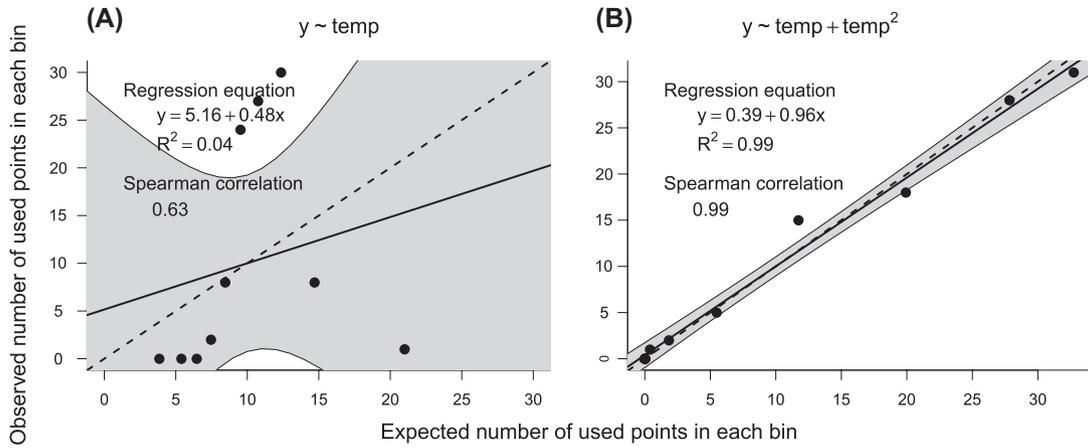


Figure 2. Presence-background binned calibration plots using the method outlined in Johnson et al. (2006) applied to simulated data for a species whose distribution was driven by temperature (x_3) and temperature². The marginal distribution of x_3 on the landscape, $f^u(x_3)$, was Normal: $f^u(x_3) = N(0,4)$. The relative probability of use (or presence) was proportional to $\exp(2x_3 - x_3^2)$. Panels depict observed versus expected numbers of presence locations within 10 bins formed using estimated relative probabilities of selection, $w(x^{test} \hat{\beta}^{train}) = \exp(x^{test} \hat{\beta}^{train})$, where x^{test} is a matrix of covariates in the test data set and $\hat{\beta}^{train}$ is a vector of regression parameter estimates obtained by fitting one of two logistic regression models to the training data (the two models are represented by the different columns). Overlaid is a regression line (black line with shaded 95% confidence intervals) relating observed and expected numbers of presence locations. A well-calibrated model should closely follow the 1:1 line (dashed line).

Let x represent the full suite of explanatory variables included in the fitted model, n_u^{test} the total number of used (i.e. presence) locations in the test data set, and z the covariates of interest (these may be covariates already included in the model or additional covariates that may be under consideration for inclusion in the model). The dimension of z may be greater than that of x , for example, if one chooses to begin with a simple model before progressively considering more complex models with additional covariates. Further, z may contain covariates that are available in the test data but are absent from the training data (e.g. if the model is applied to a new site where additional covariate data have been collected). In the example illustrated in Fig. 3, x includes only elevation, but z includes both elevation and precipitation.

1) Summarize the distribution of z at the used (i.e. presence) points in the test data set, $f^u(z)$. In our examples, we use a kernel density estimator to represent $f^u(z)$ (solid black lines/density plots in Fig. 3; Wand and Jones 1994). Similarly, summarize the distribution of z at the available (i.e. background) points in the test data set, $f^a(z)$ (dashed red lines/density plots in Fig. 3). Differences between these two densities signal that the covariate will be an important predictor of the species distribution.

2) Fit a model to the training data set. Store $\hat{\beta}$ and $c\hat{ov}(\hat{\beta})$ to characterize the uncertainty in the parameters (ignoring the intercept if using logistic regression). Assuming we have a large enough sample for $\hat{\beta}$ to be approximately normally distributed, we can draw samples from a multivariate normal distribution, $N(\hat{\beta}, c\hat{ov}(\hat{\beta}))$, to account for uncertainty in the estimated parameters. This uncertainty may alternatively be captured using a non-parametric bootstrap or via samples from a posterior distribution (if implementing the model in a Bayesian framework); bootstrapping could also be used to

account for parameter uncertainty in machine learning applications (e.g. models fit using random forests, artificial neural networks, etc.). We will refer to the distribution capturing uncertainty in $\hat{\beta}$ as the joint parameter distribution to recognize that this will be a multivariate distribution if more than one covariate is included in the model.

3) Do the following M times (with loop index i): a) to account for parameter uncertainty, select new vector of parameter values randomly from their joint parameter distribution, β^i . b) Estimate the relative probability of selection for the test data (given by Eq. (2)): $w(x^{test} \beta^i) = \exp(x^{test} \beta^i)$. c) Select a simple random sample of n_u^{test} observations from the combined (presence and background) test data, with probabilities of selection proportional to $w(x^{test} \beta^i)$ from step [3b]. d) Summarize the distribution of z associated with the points chosen in step [3c], $\hat{f}^u(z)_i$ (gray lines/density curves in Fig. 3).

4) Compare the observed distribution of covariate values at the presence points, $f^u(z)$ (black solid lines) from step [1], to the predicted distribution of these characteristics, $\hat{f}^u(z)_i$ (gray bands) from step [3], across the M simulations. One option is to overlay $f^u(z)$ (from step [1]) on a 95% simulation envelope constructed using the $\hat{f}^u(z)_i$ (Fig. 3). Alternatively, one might choose to plot the 2.5th and 97.5th quantiles of $f^u(z) - \hat{f}^u(z)_i$. We include functions in the ‘uhcplots’ package for constructing these plots and illustrate the latter type of plot in supplementary files archived with the Data Repository for the Univ. of Minnesota (Fieberg and ArchMiller 2016, Fieberg et al. 2016).

Application of UHC plots to pooled-survey data examples

To create UHC plots for the pooled-survey data examples, we constructed 1000 predicted distributions of habitat covariates

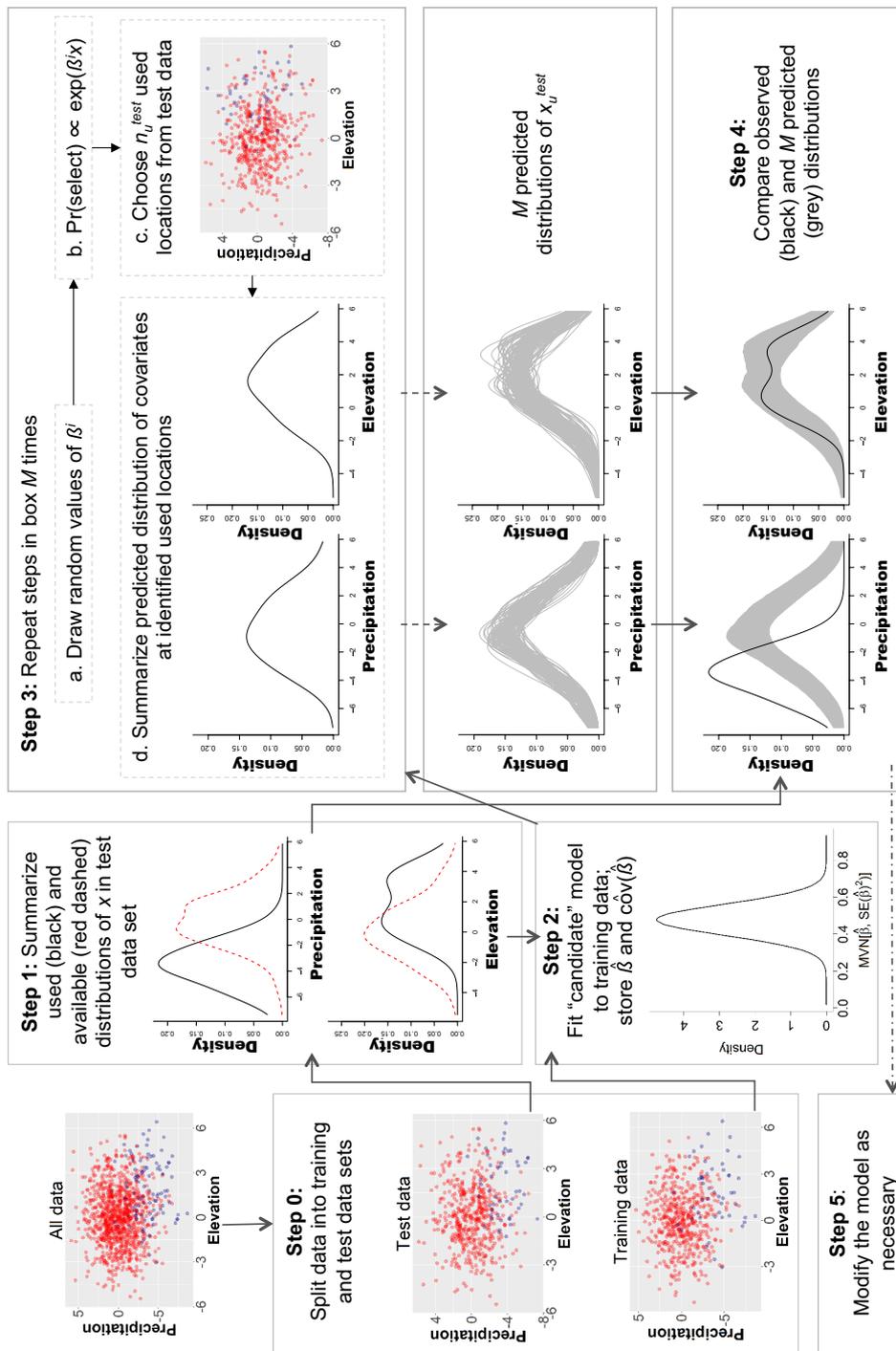


Figure 3. Steps for producing a used-habitat calibration plot. Step 0: split the data into test and training data sets (used points are shown in blue, available points in red). Step 1: summarize the distribution of the explanatory variables (here precipitation and elevation) at the presence points (solid black lines/density plots) and background points (red dashed lines/density plots) in the test data set, $f^a(x)$ and $f^b(x)$, respectively. Step 2: fit a model to the training data set, storing $\hat{\beta}$ and its uncertainty ($\text{cov}(\hat{\beta})$). In this example, the distribution of locations is driven by elevation and precipitation, but only elevation has been included in the model. Step 3: do the following M times (with loop index i): A) to account for parameter uncertainty, select new β parameter values, β^j , from the joint parameter distribution describing the uncertainty in $\hat{\beta}$; B) estimate $w(x^{test}; \beta^j) = e^{x^{test} \beta^j}$ for the test data; C) select a simple random (cross-sectional) or stratified random (step-selection function) sample of $n_{i, \text{test}}$ observations from the combined (use and available) test data, with probabilities of selection proportional to $w(x^{test}; \beta^j)$ from step [3B]; D) summarize the predicted distribution of elevation and precipitation using the points chosen in step [3C], $\hat{f}^a(x)$. Step 4: compare the observed distribution of covariate values at the used points, $f^a(x)$ from step [1], to the predicted distribution of these characteristics, $\hat{f}^a(x)$ (gray bands). Step 5: reevaluate or modify the model as necessary. In the above example, one option is to overlay $f^a(x)$ on a 95% simulation envelope constructed using the $\hat{f}^a(x)$ (gray bands). Step 5: reevaluate or modify the model as necessary. In the above example, the UHC plots would suggest that we should include precipitation in the model.

at the presence points in the test data set (i.e. $M = 1000$ in step [3]) using the models fit to the training data, accounting for uncertainty in $\hat{\beta}$ by drawing new values in each simulation from a multivariate normal distribution (the asymptotic distribution of $\hat{\beta}$; step [3a]). We compared observed (black solid lines) and predicted distributions (gray bands representing 95% simulation envelopes) of elevation and precipitation (Fig. 4) and temperature (Fig. 5) at the presence locations. We also overlaid distributions of elevation, precipitation, and temperature at the background locations, f^a (red dashed lines; Fig. 4, Fig. 5). Note that the distributions of elevation and precipitation at the presence locations (solid black lines) were shifted to the right and left, respectively, relative to the background distributions of these covariates (red dashed lines) (Fig. 4). These results reaffirm that this species tends to be found at locations with higher elevations and lower levels of precipitation. In the second example, the distribution of

temperature at the used locations was also shifted to the right relative to the background distribution (Fig. 5). In addition, the used distribution was much more peaked compared to the background distribution of temperature, which suggests that this species prefers a more narrow range of temperatures than represented by the background locations.

In the first example, the UHC plots provided evidence that the correct model with both elevation and precipitation was well-calibrated across all three data-generating scenarios (Fig. 4C–D, G–H, K–L) because the distributions of elevation and precipitation at the presence locations (solid black lines) fell mostly within the simulation envelopes generated by the fitted model (gray bands). By contrast, the elevation-only model never accurately predicted the distribution of precipitation values at the presence locations (Fig. 4B, F, J). On the other hand, it predicted the distribution of elevation at the presence locations whenever ρ_{x_1, x_2} was the same for

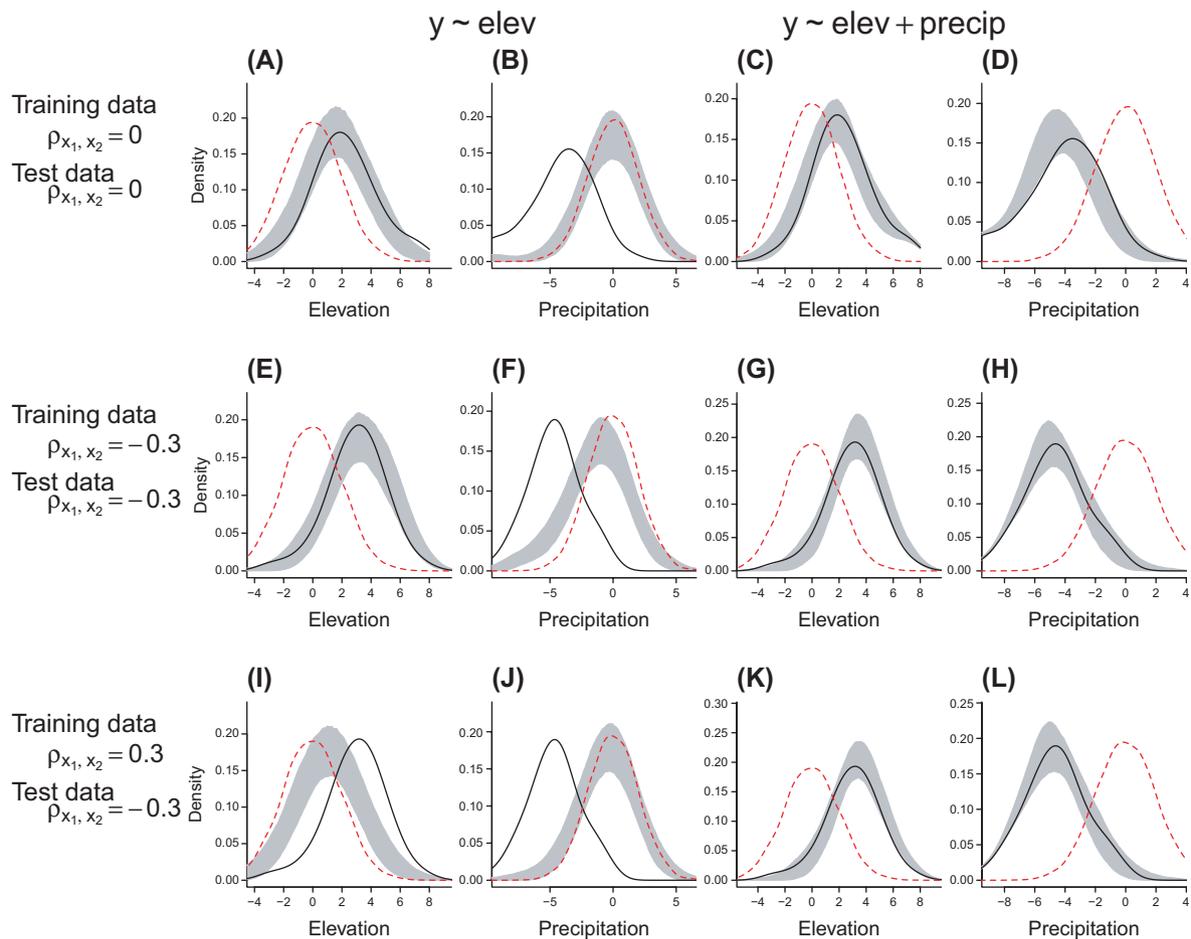


Figure 4. Used-habitat calibration (UHC) plots for the first simulation example where the species distribution was driven by elevation (x_1) and precipitation (x_2). The marginal distribution of x_1 and x_2 on the landscape, $f^a(x_1, x_2)$ (red dashed lines), was Normal: $f^a(x_1, x_2) = N(0, \Sigma)$. We considered three different data-generating scenarios in which we set $\text{var}(x_1) = \text{var}(x_2) = 4$, but varied $\text{cor}(x_1, x_2) = \rho_{x_1, x_2}$ (represented by separate rows of panels). The relative probability of use (or presence) was proportional to $\exp(0.5x_1 - x_2)$. The observed distribution of elevation and precipitation at the presence (i.e. used) points in the test data set is given by the solid black lines, with a 95% simulation envelope for these distributions given by the gray bands. Predictive distributions were formed using one of two models fit to training data, a model with elevation only (left two columns) or elevation and precipitation (the correct model; right two columns). A model is well-calibrated if the observed distributions (solid black lines) fall within the simulation envelopes.

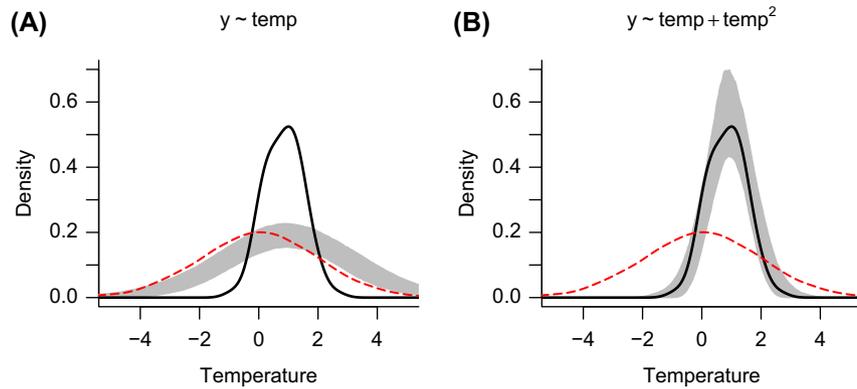


Figure 5. Used-habitat calibration (UHC) plots for the second simulation example where the species distribution was driven by temperature (x_3). The marginal distribution of x_3 on the landscape, $f^a(x_3)$ (red dashed lines), was Normal: $f^a(x_3) = N(0,4)$. The relative probability of use (or presence) was proportional to $\exp(2x_3 - x_3^2)$. The observed distribution of temperature at the presence points in the test data set is given by the solid black lines, with a 95% simulation envelope for these distributions given by the gray bands. Predictive distributions were formed using one of two models fit to training data, a model with temperature (linear term only; panel A) or temperature and temperature² (the correct model; panel B). A model is well-calibrated if the observed distributions (solid black lines) fall within the simulation envelopes.

both training and test data sets (Fig. 4A, E). Lastly, the elevation-only model failed to predict either the distribution of elevation or precipitation at the presence locations when the correlation between elevation and precipitation differed between the training and test data (Fig. 4I, J). It is worth noting that in the case where $\rho_{x_1, x_2} = -0.3$ for both training and test data sets, the elevation-only model's predictions were well-calibrated (Fig. 1C, Fig. 4E) even though the logistic regression parameter estimate for elevation was too large (0.80, SE = 0.06) relative to the data-generating value (0.5) (Table 1). These latter two results serve as a nice reminder that regression coefficients reflect partial correlations that are influenced by the suite of predictors included in the model, and are not causal effects (Fieberg and Johnson 2015). Furthermore, models may predict well in the presence of collinearity only when the correlation among predictors remains the same in training and test data (Dormann et al. 2013).

In the second simulation example, we fit a model with only a linear effect of temperature on the logit scale and another that also included a quadratic term (the correct model). When the model included only temperature, the coefficient for temperature was too low, but the coefficients were close to the data-generating values of 2 and -1 when both temperature and temperature² were included in the model (Table 2). The predicted distribution for temperature was rather broad and similar to the available distribution when only a linear effect of temperature was included in the logistic regression model (Fig. 5A). By contrast, the distribution of temperature values at presence points was rather peaked, with values of $x_1 < -2$ or > 2 rarely used (Fig. 5A). The extreme avoidance of low and high values of temperatures suggests that a quadratic effect of temperature might be needed. When we included the quadratic term for temperature in the logistic regression model, the distribution of temperature values at the observed locations fell within the 95% simulation envelope (Fig. 5B), confirming that this model was well-calibrated.

In summary, UHC plots helped to identify a missing predictor (precipitation) and also the need for a non-linear term (for temperature). It is also noteworthy that the missing predictor was identified in two scenarios where the model appeared well-calibrated when using a traditional presence-background calibration plot (Fig. 1A, C and Fig. 4B, F) (both scenarios involved predictive distributions in cases where ρ_{x_1, x_2} remained the same in training and test data sets).

Evaluating spatial predictions and model transferability

An important goal of most SDM applications is to predict species distributions in novel landscapes, which requires that models are 'transferable' to other sites, environments, and time periods. If we have location data from multiple sites, then we can evaluate transferability by fitting a model to some sites and then predicting the distribution of locations at the others (Matthiopoulos et al. 2011). UHC plots can then be used to identify areas in space where the model does a poor job of predicting. To accomplish this goal, we can include x and y spatial coordinates in z , the matrix of habitat characteristics we wish to predict at the out-of-sample used locations.

To illustrate this idea, we return to our simulation example where the species distribution was driven by elevation (x_1) and precipitation (x_2), with the probability of selecting locations proportional to $\exp(0.5x_1 - x_2)$. We simulated uniformly distributed x and y spatial coordinates for the presence and background locations associated with two landscapes (a test and a training landscape), allowing the correlation among (x, y) spatial coordinates and the habitat predictors (x_1, x_2) to differ between the two landscapes (Table 3, Fig. 6). We again fit two models to data collected from the training landscape: the first included only elevation and the second included elevation and precipitation (the correct model). We then evaluated how well these models predicted the spatial

Table 3. Correlation among spatial coordinates (x,y) and habitat covariates in training and test data in the simulation to evaluate areas in space where the model predicts poorly. The marginal distribution of elevation (x_1) and precipitation (x_2) on the landscape was given by a multivariate normal distribution with mean vector = $(0,0)$, and $\text{var}(x_1) = \text{var}(x_2) = 4$. The true species distribution was proportional to $\exp(0.5x_1 - x_2)$.

Variables	Correlation	
	Training data	Test data
x_1, x_2	0.33	0.29
x -coordinate, x_1	0.68	0.57
x -coordinate, x_2	0.33	-0.29
y -coordinate, x_1	0.35	-0.30
y -coordinate, x_2	0.67	0.57

distribution of presence points in the test landscape by creating UHC plots for the (x, y) spatial coordinates.

The presence locations in the test landscape were largely concentrated in the southeast (large x and small y ; Fig. 6). The correct model accurately predicted the distribution of (x,y) spatial coordinates (Fig. 6C, D). By contrast, the model containing only elevation resulted in a predicted distribution that was relatively uniform in space and for which the x - and y -coordinates were not well calibrated (Fig. 6A, B). This example illustrates how spatial UHC plots could be used to identify missing predictors (e.g. the poor calibration in Fig. 6A, B might lead an analyst to consider adding precipitation to the model because it follows a SE-NW gradient in the test landscape). These results also have important implications for management. In particular, one should be wary of using the elevation-only model to determine areas to conserve given

the model's poor transferability. Lastly, we note that one can use functions in the ENMeval package (Muscarella et al. 2014) to construct UHC plots with spatially-stratified cross-validation in cases where data are available from a single site. We illustrate this approach in a vignette associated with the 'uhcplots' package (Fieberg and ArchMiller 2016).

Step-selection functions

An alternative way to motivate the IPP likelihood, Eq. (1), can help with conceptualizing generalizations of this approach to longitudinal data. With telemetry data, we may consider the distribution of resources or environmental conditions at the used (i.e. presence) points, $f^u(x)$, as being selected from a distribution of values at available (i.e. background) points, $f^a(x)$, with the selection function $w(x|\beta) = \exp(x\beta)$ taking us from the distribution of available locations to the distribution of used locations by way of spatial covariates, x , and a set of regression parameters, β (Lele and Keim 2006):

$$f^u(x) = \frac{\exp(x_i\beta) f^a(x_i)}{\int \exp(x(s)\beta) f^a(x(s)) ds} \quad (3)$$

If all areas are equally available, $f^a(x(s))$ is uniform in space (and thus, a constant), getting us back to Eq. (1) (Aarts et al. 2012). Selection functions have similarly been used to correct for biased sampling procedures (Patil and Rao 1978), to study natural selection (Manly 1985), and were first introduced in the context of foraging and habitat selection by McDonald

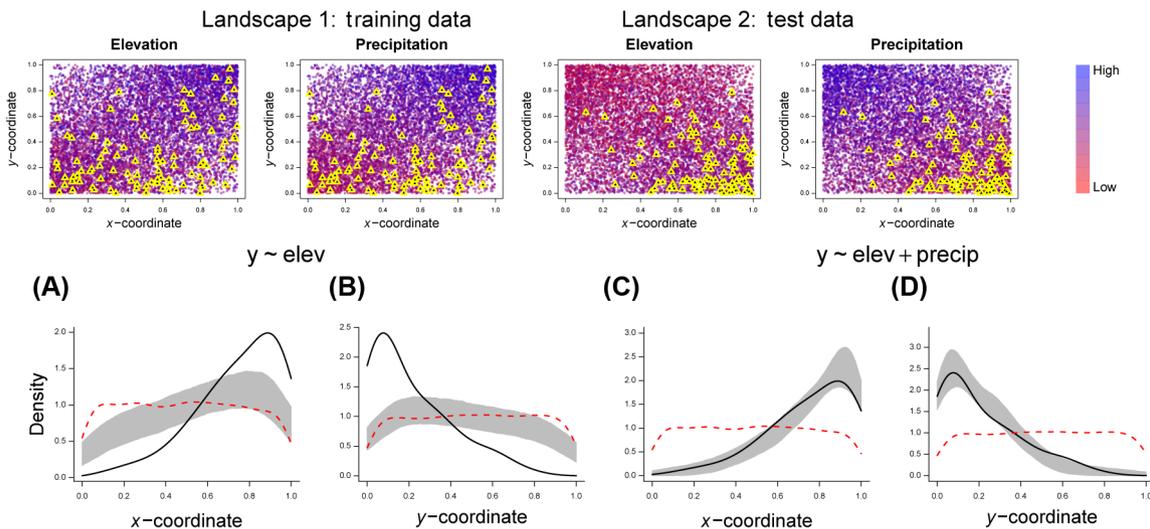


Figure 6. Used-habitat calibration (UHC) plots for spatial coordinates (x,y) . The species distribution was driven by elevation (x_1) and precipitation (x_2). The marginal distribution of x_1 and x_2 on the landscape, $f^a(x_1, x_2)$ (red dashed lines), was Normal: $f^a(x_1, x_2) = N(0, \Sigma)$. The relative probability of use (or presence) was proportional to $\exp(0.5x_1 - x_2)$. Top panels depict the background distribution of elevation and precipitation in the training and test data landscapes, with presence points overlaid in yellow and black triangles. In the bottom panels, the observed distribution of elevation and precipitation at the presence points in the test data set is given by the solid black lines, with a 95% simulation envelope for these distributions given by the gray bands. Predictive distributions were formed using one of two models fit to training data, a model with elevation only (panels A and B) or elevation and precipitation (the correct model; panels C and D). A model is well-calibrated if the observed distributions (solid black lines) fall within the simulation envelopes.

et al. (1990); the theory for estimating selection functions is well developed under the label ‘weighted distributions’ (Patil and Rao 1977).

Historically, radio-telemetry studies allowed animals to be located once to several times per day. Telemetry-based SDMs typically assumed these locations could be treated as independent, with parameters estimated by comparing these locations to randomly sampled (‘available’) sites from within an animal’s estimated home range (Fieberg et al. 2010). This approach was often justified by noting that animals had sufficient time to reach any area within their home ranges between successive locations. The advent of Global Positioning System (GPS) data and associated hardware and software now allows researchers to assess habitat use with much finer temporal resolution. As a consequence, however, telemetry locations collected close in time also tend to be close in space, and the only sites available to an animal shortly after one observation are those accessible to the animal from the previous location, within the time step.

Step-selection functions were developed to address these concerns (Fortin et al. 2005, Forester et al. 2009, Avgar et al. 2016). Rather than treat locations as independent and assume a uniform distribution for $f^a(x)$, step-selection functions treat movements between locations as independent. Background locations specific to each telemetry location are generated by considering the previous location, the time between successive locations, and the movement characteristics of the study species – in particular, step lengths (distances between consecutive points collected at fixed temporal intervals) and turn angles (change in bearing between consecutive locations) (Thurfjell et al. 2014, Avgar et al. 2016). Background locations are generated by sampling step lengths and turn angles from their empirical distributions (Fortin et al. 2005) or from appropriate statistical distributions (e.g. exponential or gamma for step length, von Mises for turn angles) (Forester et al. 2009, Avgar et al. 2016). Step lengths and turn angles are then combined with the location at the previous time point to generate possible movement paths, and as a result, distributions of available points that are location-specific. To guard against misspecification of the step length and turn angle distributions (or, alternatively, to estimate parameters in assumed statistical distributions describing these movement characteristics), one can include as covariates various functions of the distance between points and angular deviations from the previous step (Forester et al. 2009, Avgar et al. 2016).

The likelihood for these data is similar to that for the inhomogeneous Poisson process model, except that we now have stratified data (one stratum for each observed location and its associated available locations generated by the random movement paths):

$$L(y_i | x_i, \beta) = \prod_{i=1}^K \frac{\exp(x_{i(k)}\beta)}{\sum_{j=1}^{n_i} \exp(x_{j(k)}\beta)} \quad (4)$$

where K is the number of strata, n_i is the number of locations (used plus available) in stratum i , and $x_{j(k)}$ are the covariates

associated with the j^{th} point in the k^{th} stratum (with $x_i(k)$ giving the covariates for the used location).

Calibration plots with step-selection functions

It is unclear how traditional presence-background calibration plots (Boyce et al. 2002, Johnson et al. 2006, Phillips and Elith 2010) might be adapted to step-selection functions. In particular, it is not clear how we should account for the strata, which contain a fixed number of used locations (usually one). By contrast, UHC plots can be adapted to step-selection functions with only two minor changes: 1) rather than fit a logistic regression model in step [2], we can fit a conditional logistic regression model; 2) rather than select a simple random sample in step [3c], we can select a stratified random sample (i.e. selecting one point from within each stratum). No other modifications are necessary.

Here, we illustrate the application of UHC plots to step-selection functions fit to moose *Alces alces* telemetry data. From 2010–2015, technicians captured 170 adult female moose in northeastern Minnesota. Technicians fitted moose with Iridium GPS radiocollars (VECTRONIC Aerospace, Berlin, Germany) recording animal locations at 4.25, 2, and 1.065-h fix rates. For a full description of capturing and deployment protocols see Carstensen et al. (2014). We selected a single animal with data from summer 2013 and summer 2014 and subsampled data collected at higher fix rates to achieve a consistent 4.25-h fix rate ± 0.25 h. We excluded fixes within 24 h of deployment and those with horizontal dilution of precision > 10 (Rempel and Rodgers 1997). This left a total of 689 used locations in both 2013 and 2014.

We generated 10 available locations for each used location by randomly selecting 10 step lengths and 10 turn angles to project the animal forward in time from the previous location (see Street et al. 2016 for full description of data development). We defined resource availability at used and available locations as the proportional cover of four land cover types within a 50 m radius buffer (identified in the National Land Cover Database 2011; Jin et al. 2013): deciduous forest (decid50), mixedwood forest (mixed50), coniferous forest (conif50) and treed wetlands (treedwet50).

We fit three conditional logistic regression models to the moose data using the ‘clogit’ function in the survival package of Program R (R Core Team, Therneau 2015), treating locations from 2013 as training data and locations from 2014 as test data. In the first model, we included decid50, mixed50, conif50, and treedwet50 as explanatory variables. In the second model, we included the same set of predictors, except we dropped mixed50. Lastly, we fit a model containing only mixed50. We also included step length (divided by 1000 to scale the magnitude of the regression coefficient to that of the land cover classes) in each of the models to accommodate bias introduced by using parametric distributions for generating step-lengths (Forester et al. 2009, Avgar et al. 2016).

In the original step-selection model, the coefficient for conif50 was negative, whereas the coefficients for decid50, mixed50, and treedwet50 were all positive; of these, only the coefficient for mixed50 was statistically significant (Table 4).

Table 4. Parameter estimates (SE) from step-selection functions fit to moose *Alces alces* data in Minnesota using conditional logistic regression. Covariates measured the proportional cover of 4 land cover types within a 50 m radius buffer: deciduous forest (decid50), mixedwood forest (mixed50), coniferous forest (conif50), and treed wetlands (treedwet50). We also included step length (divided by 1000 to scale the magnitude of the regression coefficient to that of the land cover classes; step) to accommodate bias introduced by using parametric distributions for generating step-lengths.

Variable	Model		
	(1)	(2)	(3)
decid50	0.49 (0.33)	-0.60 (0.19)	
mixed50	1.38 (0.24)		1.03 (0.16)
conif50	-0.30 (0.38)	-1.37 (0.27)	
treedwet50	0.40 (0.31)	-0.70 (0.16)	
step	-6.33 (0.25)	-6.44 (0.25)	-6.39 (0.25)

When we dropped mixed50 from the model, the coefficients in the step-selection function changed drastically; the coefficients for decid50 and treedwet50 even changed sign (Table 4). The coefficients for all of the compositional predictors left in the model were negative (and all statistically significant), which likely reflects the fact that having more of any one of these habitat types within 50 m meant having less of mixed50. This series of models nicely illustrates some of the challenges involved with modeling compositional data due to multicollinearity among the predictors (Graham 2003, Cade 2015).

To produce UHC plots for these models, we again simulated 1000 used test data sets, drawing new regression parameters each time from $N(\hat{\beta}, \text{cov}(\hat{\beta}))$. The UHC plots were similar for all three models, with the distribution of the covariates at the used points in the test data set largely falling within the predicted distributions for each of the explanatory variables (Fig. 7). These plots suggest that the models are well-calibrated, but also that the information about selection can be captured by a single compositional predictor, mixed50 (Fig. 7I–L).

Discussion

The combination and popularity of open source software (Ghisla et al. 2012, R Core Team), remote sensing technologies, and a plethora of modeling approaches has facilitated the application of models linking plant and animal locations to environmental variables. Further, geographic information systems (GIS) make it easy to produce maps depicting predicted distributions for sampled and unsampled areas. But, how good are these models and the maps they produce? Should we trust models to predict distributions in novel environments, particularly when they are constructed by considering a large suite of often multicollinear predictors (Dormann et al. 2013)? These questions are of utmost

importance to wildlife managers and conservation biologists, and thus it is not surprising that they have garnered significant attention lately from ecologists working across a wide range of taxa (Vanreusel et al. 2007, Moreno-Amat et al. 2015, Torres et al. 2015, Duque-Lazo et al. 2016, Huang and Frimpong 2016).

Most popular approaches to fitting species distribution or habitat selection models rely on comparing observed locations of individuals to randomly or systematically selected locations that describe the background distribution or availability of resources or environmental conditions. Frequently, the combined presence-background data are modeled using binary regression models, with $Y_i = 1$ for observed locations and 0 for background locations (Johnson et al. 2006, Fithian and Hastie 2013). This treatment of the data originally led to much concern and confusion among practitioners who recognized that background points (with $Y_i = 0$) might actually be used by the species (Keating and Cherry 2004). Recent connections between common modeling approaches (e.g. MaxEnt, spatial logistic regression) and inhomogeneous Poisson process models have clarified both the role of the background points (they serve as quadrature points in Eq. (1); Warton and Shepherd 2010) and also the interpretation of regression parameters (they describe systematic variation in the log intensity of the Poisson process model; Aarts et al. 2012, Fithian and Hastie 2013, Renner et al. 2015).

As more researchers become aware of these connections, we expect to see a similar paradigm shift in terms of the methods proposed for validating species distribution and habitat selection models. Traditionally, methods for validating species distribution models have mimicked or modified approaches developed for presence-absence data. They have treated the number of presence locations as random, and have focused on how well the models do at predicting whether locations are ‘used’ or ‘available’. By contrast, UHC plots consider the number of presence locations as fixed, and instead focus on validating a model’s ability to predict the characteristics (i.e. the biotic and abiotic factors used to model distribution patterns) at these locations using out-of-sample data. Our simulation examples demonstrated the utility of UHC plots for identifying missing covariates and nonlinearities that should be included in the model as well as how these plots can be used to identify areas in space that are poorly predicted. Our empirical example, based on moose movement data, demonstrated how this approach can accommodate the stratified nature of step-selection functions and, further, how UHC plots can be used to provide insights into the effect of multicollinearity, particularly when considering compositional data. Future work should focus on exploring the use of UHC plots to suggest possible transformations (e.g. log, step functions) or to detect other forms of model misspecification (e.g. the need for interactions). Simulated data are critical to these efforts since they allow one to evaluate model performance in scenarios where the factors driving the underlying species distribution are known (Miller 2014, Leroy et al. 2016).

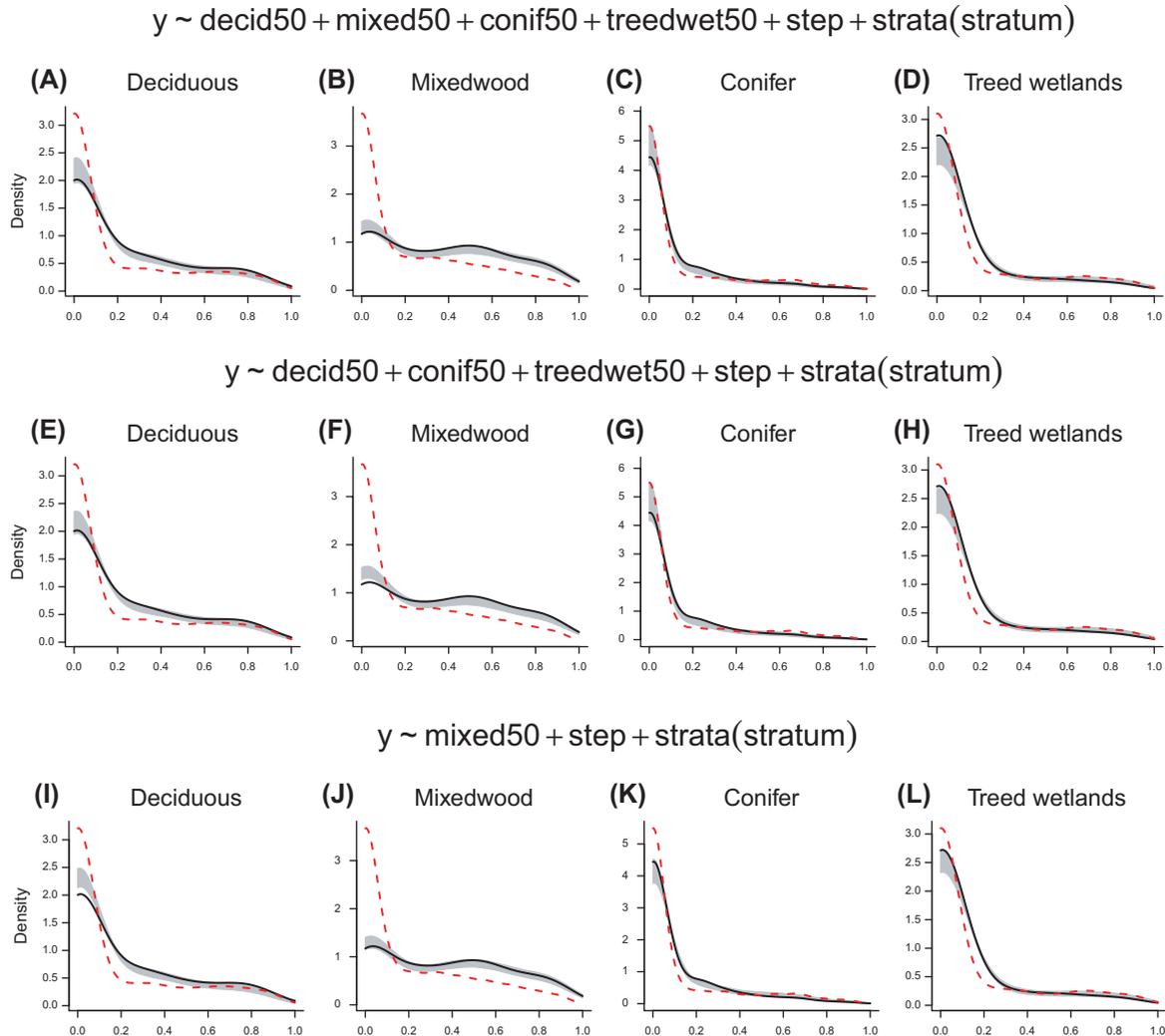


Figure 7. Used-habitat calibration plots for step-selection models fit to moose *Alces alces* data in Minnesota. We considered three different models (represented by the three rows of panels), each containing a different subset of covariates (as indicated above each row of panels). Covariates in the models measured proportional coverage of deciduous forest (decid50), mixedwood forest (mixed50), conifer forest (conif50), and treed wetland (treedwet50) within a 50 m buffer of each location. We also included step length (divided by 1000 to scale the magnitude of the regression coefficient to that of the land cover classes; step) to accommodate bias introduced by using parametric distributions for generating step-lengths. Panels depict the distribution of available and used locations in the test data set (red dashed and solid black lines, respectively), along with 95% simulation envelopes for the predicted distribution of these habitat covariates at the used locations from the fitted step-selection functions. A model is well-calibrated if the observed distributions (solid black lines) fall within the simulation envelopes.

Recently developed approaches for assessing fit of spatial point process models offer another promising alternative to UHC plots considered here (Baddeley et al. 2005, 2013, Renner et al. 2015). Specifically, one can plot residuals against spatial covariates or smoothed residuals versus spatial location (e.g. easting, northing). These types of plots are available in the ‘spatstat’ library of Program R and have a strong theoretical basis (Baddeley et al. 2008). The advantage of the approach we suggest is that it can be applied more generally, as we have demonstrated with fitted logistic regression models and step-selection functions. The ability to construct simulation envelopes for out-of-sample data is

another advantage, especially since most applications of species distribution models consider a large suite of explanatory variables and often allow for considerable model complexity, leading to data-driven models that may be overfit and perform poorly when applied to new data (Giudice et al. 2012, Harrell 2013).

Understanding what motivates animals to move from one location to another, and how the broad-scale patterns of resources and risk affect the distribution of a species in the landscape is of critical importance to the management and conservation of wildlife and plant species. For models of species distributions to be useful, they must be more

than shots in the dark. They must be able to make predictions about how a species will respond to new environmental conditions presented at different locations in space and time in the face of anthropogenic landscape change. By comparing model predictions to out-of-sample data, UHC plots can identify important features that are well-predicted and others where improvement is needed. This process can shed light on how best to modify models, provide important insights into factors driving the distribution of species, and ultimately enhance the reliability and generality of conservation and management recommendations.

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Combinations of Abiotic Factors Differentially Alter Production of Plant Secondary Metabolites in Five Woody Plant Species in the Boreal-Temperate Transition Zone

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Plant secondary metabolites (PSMs) are a key mechanism by which plants defend themselves against potential threats, and changes in the abiotic environment can alter the diversity and abundance of PSMs. While the number of studies investigating the effects of abiotic factors on PSM production is growing, we currently have a limited understanding of how combinations of factors may influence PSM production. The objective of this study was to determine how warming influences PSM production and how the addition of other factors may modulate this effect. We used untargeted metabolomics to evaluate how PSM production in five different woody plant species in northern Minnesota, United States are influenced by varying combinations of temperature, moisture, and light in both experimental and natural conditions. We also analyzed changes to the abundances of two compounds from two different species – two resin acids in *Abies balsamea* and catechin and a terpene acid in *Betula papyrifera*. We used permutational MANOVA to compare PSM profiles and phytochemical turnover across treatments and non-metric multidimensional scaling to visualize treatment-specific changes in PSM profiles. We used linear mixed-effects models to examine changes in phytochemical richness and changes in the abundances of our example compounds. Under closed-canopy, experimental warming led to distinct PSM profiles and induced phytochemical turnover in *B. papyrifera*. In open-canopy sites, warming had no influence on PSM production. In samples collected across northeastern Minnesota, regional temperature differences had no influence on PSM profiles or phytochemical richness but did induce phytochemical turnover in *B. papyrifera* and *Populus tremuloides*. However, warmer temperatures combined with open canopy resulted in distinct PSM profiles for all species and induced phytochemical turnover in all but *Corylus cornuta*. Although neither example compound in *A. balsamea* was influenced by any of the abiotic conditions, both compounds in *B. papyrifera* exhibited

115 significant changes in response to warming and canopy. Our results demonstrate 172
116 that the metabolic response of woody plants to combinations of abiotic factors 173
117 cannot be extrapolated from that of a single factor and will differ by species. This 174
118 heterogeneous phytochemical response directly affects interactions between plants and 175
119 other organisms and may yield unexpected results as plant communities adapt to novel 176
120 environmental conditions. 177
121 178

122 **Keywords: phytochemical turnover, PSM diversity, untargeted metabolomics, balsam fir, beaked hazel, paper 179**
123 **birch, red maple, trembling aspen 180**
124 181
125 182

126 INTRODUCTION 183

127
128 Plant secondary metabolites (PSMs) are one of the primary 184
129 ways in which plants respond to environmental variability, and 185
130 regulation of PSM production is strongly influenced by the local 186
131 environment (Wink, 1988; Bennett and Wallsgrave, 1994; Bray 187
132 et al., 2000; Hirt and Shinozaki, 2003). Many interactions between 188
133 plants and other organisms are mediated by PSMs (Farmer, 2001; 189
134 Karban et al., 2006; Karban, 2008), and thus, the biochemical 190
135 mechanisms that influence these interactions are modulated, at 191
136 least in part, by the presence, absence, or magnitude of various 192
137 environmental factors (DeLucia et al., 2012; Jamieson et al., 193
138 2012). For instance, changes in the amount and seasonality of 194
139 precipitation have been shown to influence concentrations of 195
140 cyanogenic glycosides (Gleadow and Woodrow, 2002; Vandegeer 196
141 et al., 2013), and elevated concentrations of atmospheric CO₂ 197
142 often result in increased concentrations of condensed tannins 198
143 (Lindroth, 2012). Evidence is mounting that recent warming may 199
144 also influence the production of PSMs (Kuokkanen et al., 2001). 200

145 Studies investigating the influence of warming on PSM 201
146 production suggest that temperature-induced changes to PSMs 202
147 may be species, compound, or even context dependent. For 203
148 example, warming has been shown to have no effect on levels 204
149 of phenolics in red maple (*Acer rubrum*, Williams et al., 2003), 205
150 Norway spruce (*Picea abies*, Sallas et al., 2003), and Scots pine 206
151 (*Pinus sylvestris*, Sallas et al., 2003) but resulted in decreased levels 207
152 of phenolics in dark-leaved willow (*Salix myrsinifolia*, Veteli 208
153 et al., 2006) and silver birch (*Betula pendula*, Kuokkanen et al., 209
154 2001). Additionally, warming has been shown to increase levels of 210
155 terpene-based compounds in Norway spruce (Sallas et al., 2003), 211
156 Ponderosa pine (*Pinus ponderosa*, Constable et al., 1999), and 212
157 Scots pine (Sallas et al., 2003) but has been shown to both increase 213
158 (Constable et al., 1999) and decrease (Snow et al., 2003) levels of 214
159 monoterpenes in Douglas fir (*Pinus menziesii*). While evidence of 215
160 warming-induced changes to phytochemistry is important to our 216
161 understanding of how plants will respond to future climates, in 217
162 natural settings, elevated temperature often combines with other 218
163 abiotic conditions to influence PSM production and potentially 219
164 modulate any changes to phytochemistry that might otherwise be 220
165 induced by warming alone. 221

166 As temperatures continue to rise, global precipitation patterns 222
167 are expected to shift (Hurrell, 1995; Alexander et al., 2006; IPCC, 223
168 2014) and light availability to understory plants will likely be 224
169 altered due to changes in the frequency and intensity of forest 225
170 disturbance patterns (Canham et al., 1990; Dale et al., 2001). 226
171 While variability in each of these environmental factors has 227
228

183 been shown to influence production of PSMs on their own 184
185 (Bryant et al., 1983; Dudd and Shure, 1994; Pavarini et al., 2012), 186
187 combinations of factors can have a distinct effect (Rizhsky et al., 188
189 2002, 2004; Mittler, 2006; Zandalinas et al., 2018). Moreover, 190
191 plant responses to combinations of abiotic factors can be either 192
193 synergistic or antagonistic (Bonham-Smith et al., 1987; Mittler, 194
195 2006; Zandalinas et al., 2018). For example, drought has been 196
197 shown to enhance cold tolerance (Cloutier and Andrews, 1984), 198
199 but also exacerbate a plant's intolerance of high temperatures 200
201 (Rizhsky et al., 2002). Further, different combinations of salinity 202
203 and high temperatures have been shown to have both positive and 204
205 negative influences on the metabolism of reactive oxygen species 206
207 and stomatal response (Zandalinas et al., 2018). Regardless, 208
209 the vast majority of current research remains focused on the 209
210 influences of individual conditions rather than considering 210
211 potential interactions among them. 211
212

213 Until recently, the majority of studies investigating the 214
215 potential influence of different abiotic factors largely considered 216
217 the effects of these factors on individual compounds or small 217
218 groups of compounds. However, individual metabolites rarely, 218
219 if ever, function in isolation (Gershenson et al., 2012). Rather, 219
220 the influence of any one compound is dependent on conditions 220
221 within the local environment, as well as the relative abundance 221
222 of numerous other metabolites within a plant's array of chemical 222
223 constituents (Dyer et al., 2003; Richards et al., 2010; Gershenson 223
224 et al., 2012; Jamieson et al., 2015). Thus, understanding how 224
225 changes in the abiotic environment will influence a plant's 225
226 metabolic profile is important for interpreting how these changes 226
227 will influence the abundance and biological role of individual 227
228 compounds as well. 228

213 Phytochemical diversity influences how effective plants are 214
215 when defending against a range of threats (Gershenson et al., 214
216 2012; Frye et al., 2013; Richards et al., 2015). Compounds may 215
216 act synergistically, thereby forming mixtures that can provide 216
217 enhanced protection against potential hazards (Gershenson, 217
218 1984; Harborne, 1987; Gershenson et al., 2012). Indeed, recent 218
219 evidence suggests that the number of individual compounds 219
220 comprising a plant's phytochemical profile can even influence 220
221 local biological diversity via the influence of changes in toxicity 221
222 on rates of herbivory (Richards et al., 2015). Increased diversity 222
223 of secondary metabolites may also allow for more precise 223
224 communication between plants, thereby allowing for more robust 224
225 protection against a range of conditions (Iason et al., 2005; 225
226 Poelman et al., 2008; Gershenson et al., 2012; Frye et al., 226
227 2013). Two metrics that are useful for assessing changes in 227
228 phytochemical diversity are "phytochemical richness" (i.e., the 228

absolute number of compounds produced) and “phytochemical turnover” (i.e., the degree of overlap among the compounds produced), as both measures provide different insights into the metabolic response of plants to a range of abiotic conditions.

Variability in phytochemistry, even within the same species, may influence ecosystem structure and function through an array of chemically driven ecological effects (Bukovinszky et al., 2008; Gillespie et al., 2012; Sedio et al., 2017). The growth-differentiation balance hypothesis (GDBH) suggests that as the local environment becomes increasingly stressful, growth processes will become limited and the production of PSMs will increase until the point that PSM production also becomes limited by resource acquisition/availability (Lerdau et al., 1994). While phytochemical diversity has not been explicitly tested in light of the GDBH, studies have shown that herbivore-induced secondary chemistry can be completely suppressed in some woody species under a range of abiotic conditions (Lewinsohn et al., 1993), rendering them vulnerable to further invasion by pests and pathogens. While the number of studies investigating the effects of warming and other abiotic conditions on PSM production is rapidly growing, we currently have a limited understanding of how different abiotic factors may interact to influence phytochemical diversity (Bidart-Bouzat and Imeh-Nathaniel, 2008; Jamieson et al., 2012, 2015). The objective of this study was to determine how elevated temperatures may influence the production of PSMs and to evaluate how the addition of other abiotic factors may modulate this effect.

While a targeted approach uses standard model compounds to identify and observe changes in specific compounds selected a priori, an untargeted (i.e., global) approach makes no assumptions regarding specific metabolites, and therefore, allows one to observe global changes across the entire metabolic profile. Thus, the strength of an untargeted approach lies in the potential to discover unanticipated changes in metabolic profiles as a result of environmental perturbations (Crews et al., 2009). Although untargeted metabolomics have been used in medicine for clinical diagnosis of various diseases, including numerous forms of cancer (Sreekumar et al., 2009; Jain et al., 2015), this study is among the first to apply this method to an ecological setting.

We used an untargeted metabolomics approach to evaluate how the phytochemical profiles of five different woody plant species are influenced by temperature, soil moisture, and light. Specifically, we tested the hypothesis that elevated temperatures alter the production of PSMs by leading to phytochemical profiles that are distinct from those found at ambient temperature (H1) and that warming will change phytochemical diversity via reductions in phytochemical richness or a high degree of turnover (H2). We also tested the hypothesis that the addition of other abiotic factors, specifically high light and drought, will either magnify or nullify temperature-induced changes in phytochemical profiles and PSM diversity (H3). Finally, because individual compounds may vary greatly in response to heterogeneity in the abiotic environment, we identified two ‘example compounds’ from balsam fir (*Abies balsamea* – two unspecified di-terpene resin acids) and paper birch (*Betula papyrifera* – catechin and another unspecified di-terpene resin acid) and analyzed the effects of different sets of

abiotic factors (high-temperature, light, and drought) on their relative abundance. Specifically, we tested the hypothesis that individual compounds will respond to different conditions and combinations of conditions by either increasing or decreasing in relative abundance, potentially in a non-uniform and unpredictable manner (H4).

MATERIALS AND METHODS

Experimental Design

The Boreal Forest Warming at an Ecotone in Danger (B4WarmED) project is an ecosystem experiment that simulates both above- and below-ground warming in a boreal forest community. The experiment was conducted at Cloquet Forestry Center (CFC; Cloquet, MN, United States) and was initiated in 2008. The experimental design consists of a 2 (overstory – open and closed) \times 3 (warming – ambient, ambient +1.7°C, and ambient +3.4°C) \times 2 (precipitation – ambient and ambient –40%) factorial design with six replicates (two per block) per treatment combination, for a total of 72 – 7.1 m² plots (Rich et al., 2015). Within each plot, 121 seedlings of 11 tree species were planted into the remaining herbaceous vegetation in a gridded design (Rich et al., 2015). Above-ground biomass was warmed using a Temperature Free-Air-Controlled Enhancement System (T-FACE) and below-ground biomass was warmed via buried resistance-type heating cables (Rich et al., 2015). Above- and below-ground temperatures have been monitored and logged at 15-min intervals since spring 2008. In 2012, event-based rain enclosures were installed on nine plots in the open overstory replicates of the warming experiment, which allowed for safe and reliable removal of rainfall. Mean annual rainfall exclusion from June to September ranges from 42 to 45%.

We collected plant samples from the B4WarmED project during two different time periods. On July 14, 2013, we collected samples of balsam fir and paper birch that were grown under closed overstory and three warming treatments, and on July 15, 2014, we collected samples of balsam fir, paper birch, trembling aspen (*Populus tremuloides*), and red maple (*Acer rubrum*) grown under open overstory in the three warming treatments and two precipitation treatments. Where possible, we collected recent-growth foliar tissue from two plants per species within each replicate plot. However, some replicates contained either one or no plants with enough leaf tissue to sample. Samples sizes were particularly small during 2014, so we were forced to group individual warming treatments (ambient, +1.7°C, +3.4°C) into a binary response (ambient temperature vs. elevated temperature). All plant samples were collected within a 2-h time period. Upon collection, samples were flash frozen with dry ice, and subsequently stored in a –80°C freezer to minimize chemical degradation. We broadly refer to samples collected from the B4WarmED project as our “experimental” samples.

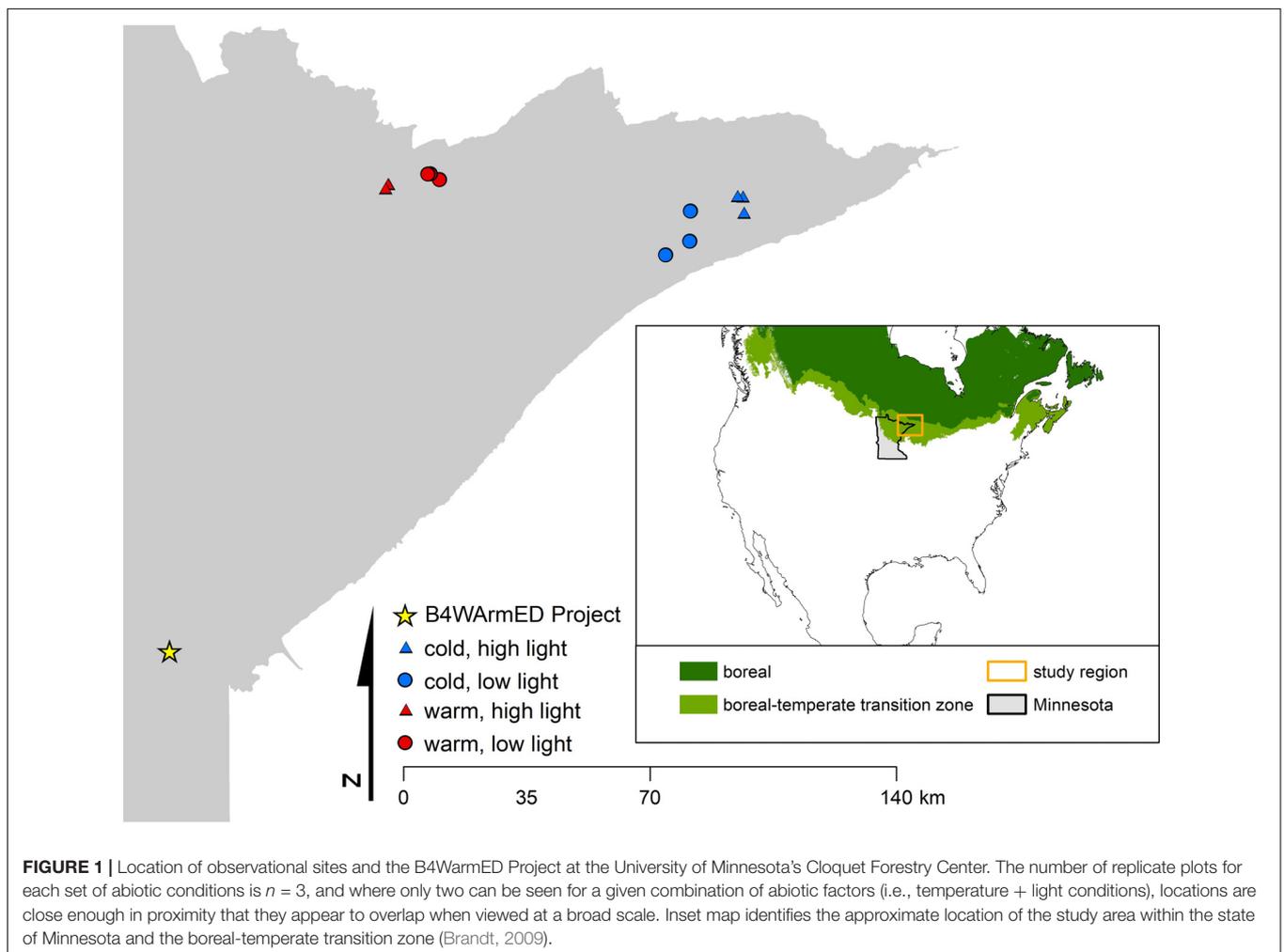
To investigate how temperature and light conditions may interact to influence phytochemical production in a natural forest environment, we collected samples of balsam fir, paper birch, trembling aspen, and beaked hazel (*Corylus cornuta*) from open and closed canopy environments across two regions

343 in northeastern Minnesota (Figure 1). These regions exhibit
 344 differences in mean-maximum summer temperature (maximum
 345 daily temperature averaged across June, July, and August) of
 346 approximately 5.5°C (Supplementary Table S1). On July 14,
 347 2015, we collected a minimum of 3 biological replicates from each
 348 species within each set of abiotic conditions. The sampling design
 349 consists of a 2 (overstory – open and closed) × 2 (temperature –
 350 warm and cool) design with three plot replicates per treatment
 351 combination, for a total of 12 – 400 m² plots. Open-canopy plots
 352 allowed us to evaluate high-light conditions on production of
 353 PSMs and were located in areas that were clear-cut in 2006 (i.e.,
 354 open overstory), while closed-canopy plots were located in areas
 355 that experienced no known overstory disturbance since at least
 356 1985 (i.e., closed overstory). Thus, light conditions for all plots
 357 were based on whether the overstory was open (i.e., high light)
 358 or closed (i.e., low light). Temperature logger data collected for a
 359 parallel study from similar plot types suggest that average high
 360 temperatures from May 1, 2015 to July 14, 2015 ranged from
 361 30.4°C in low-light plots in the cool region to 36.6°C in high-light
 362 plots in the warm region. All field samples were collected on the
 363 same day, within an 8-h period. Upon collection, samples were
 364 flash frozen with dry ice, and subsequently stored in a –80°C

freezer. For brevity, we occasionally refer to samples collected
 throughout northeast Minnesota as “observational” samples.

Study organisms

Balsam fir is a mid- to large-sized species of conifer, growing
 to 26 m in height, with shallow roots (Smith, 2008). It is highly
 vulnerable to drought, fire, and spruce budworm (*Choristoneuro
 fumiferana*) infestations (Engelmark, 1999), and modest climate
 warming has been shown to decrease net photosynthesis and
 growth by as much as 25% (Reich et al., 2015). Paper birch
 can grow to 28 m in height (Smith, 2008) and is drought
 and shade intolerant (Iverson and Prasad, 1998; Iverson et al.,
 2008). While it can grow rapidly and live to be 250 years
 of age, seedlings need significant light to prosper (Kneeshaw
 et al., 2006). Elevated temperatures have been shown to influence
 foliar nitrogen, lignin, and condensed tannins in both paper
 birch and trembling aspen with the specific response varying
 as a function of species and climate (Jamieson et al., 2015).
 Trembling aspen is one of the most widespread tree species in
 North America and occurs on a wide-range of soil types and
 in various climatic conditions (Smith, 2008). It is sensitive to
 both drought and shade (Iverson and Prasad, 1998; Iverson et al.,



2008) and may become increasingly vulnerable to other potential stressors under conditions of drought and high temperatures (Worrall et al., 2008). Red maple is a moderately large tree, growing to 29 m in height and is known to be tolerant to a wide-range of precipitation conditions, from drought to seasonal flooding (Smith, 2008). While this species is expected to prosper under future climate scenarios (Iverson and Prasad, 1998; Iverson et al., 2008) and performed well under experimental warming (Reich et al., 2015), both prolonged flooding and severe drought have been shown to result in senescence and decreased growth, respectively (Nash and Graves, 1993). Beaked hazel, a shade-tolerant shrub that can grow to 4 m tall, is a common understory species in both conifer and deciduous forests and occurs almost exclusively in fire prone habitats (Smith, 2008). Beaked hazel is highly sensitive to fire and previous work suggests that growth may be limited by soil moisture (Johnston and Woodard, 1985).

Metabolite Analysis

Tissue samples were lyophilized for 72 h and then homogenized and extracted using 25 mg (+/-2.5 mg) of each sample. Homogenization and extraction were performed for 5 min at a frequency of 1500 Hz with 1 ml of 70% isopropyl alcohol at -20°C using a bead mill and 2.8 mm tungsten carbide beads (Sped Sample Prep GenoGrinder 2010, Metuchen, NJ, United States). Samples were then subjected to centrifugation at 16,000 × g for 5 min. The supernatant was then removed and subjected to an additional centrifugation step, 16,000 × g for an additional 5 min, and the supernatant was collected for subsequent analysis. Finally, 20 µL of each supernatant sample was removed and pooled to use as a control. All samples were then stored at -80°C.

We analyzed samples with liquid chromatography mass spectrometry (LC-MS) using an Ultimate 3000 UHPLC (ultra-high-performance liquid chromatography) system coupled to a Q Enactive hybrid quadrupole-Orbitrap mass spectrometer with a heated electrospray ionization (HESI) source (Thermo Fisher Scientific, Bremen, Germany). We injected 1 µL of each sample per analysis onto an ACQUITY UPLC HSS T3 column, 100 Å, 1.8 µm, 2.1 mm × 100 mm (Waters, Milford, MA, United States) using a gradient composed of solvents A: 0.1% formic acid and B: acetonitrile. Specifically, 0–2 min, 2% B; 6 min, 24% B; 9 min, 33% B; 12 min, 65% B; 16 min, 80% B; 20 min 93% B; 21 min 98% B; 22 min 98% B; 23 min 2% B; 23–25 min 2% B. Samples were analyzed in a randomized order to minimize systematic bias from instrument variability and carryover. Full-scan analysis was performed using positive/negative ion polarity switching, a 115–1500 *m/z* scan range, a resolution of 70,000 (at *m/z* 200), maximum fill times of 100 ms, and target automatic gain control (AGC) of 1 × 10⁶ charges. Ion fragmentation was performed using a higher-energy collision dissociation (HCD) cell and resulting MS/MS data were collected using a resolution of 17,500, maximum fill times of 100 ms, and an AGC target of 2 × 10⁵ charges. Normalized collision energies (NCE) ranged from 10 to 45 in increments of 5. All data were collected using Xcalibur version 2.2 (Thermo Fisher Scientific, Bremen, Germany).

Example Compounds

To determine which chemical features varied consistently and significantly among each treatment and species group, we aligned, smoothed, background subtracted, and analyzed all chromatographic data using analysis of variance ($\alpha = 0.001$) via Genedata 7.1 (Genedata, Basel, Switzerland). We assigned putative metabolite identities only to the features found to be significantly abundant (ANOVA, $\alpha = 0.001$) with an exact mass and higher-energy collisional dissociation (HCD) MS/MS fragmentation spectra. We determined molecular formulae by using exact mass to calculate the most probable elemental composition for each feature (Supplementary Table S2). We then manually interpreted HCD spectra collected at numerous collision energies (Supplementary Figures S1–S3), and compared these to the MassBank database using MetFusion (Gerlich and Neumann, 2013). Where possible, we confirmed the identity of individual compounds via comparison to an authenticated standard (Sigma-Aldrich) and assigned other putative identities by matching molecular formulae to those of previously observed metabolites in *Betula* (Julkunen-Tiitto et al., 1996) and *Abies* (Otto and Wilde, 2001). Specifically, we analyzed changes in the relative abundance of catechin and an unspecified terpene acid in paper birch and two unspecified diterpene resin acids in balsam fir. The identification of catechin was confirmed by comparison of accurate mass, LC-retention and MS/MS fragmentation properties of commercially available standard compounds for both catechin and its frequently associated isomer epicatechin which were distinguishable by chromatographic separation. There has been a great deal of work investigating the biological and ecological activity of catechin and terpenoid-based metabolites (Tahvanainen et al., 1985; Gershenson and Croteau, 1992; Berg, 2003; Stolter et al., 2005); and as a result, we expect our results regarding these compounds to be broadly relevant.

Data Processing and Statistical Analysis

Data processing and statistical analyses were conducted using R 3.5.0 (R Core Team, 2017). To initiate data processing, we used the *xcmsRaw* function in the *xcms* package (Smith et al., 2006; Tautenhahn et al., 2008; Benton et al., 2010) to read our raw mzML files into R. After separating our data by polarity using the *split* function in the *base* package, we used the *findPeaks.centwave* function for peak detection, which we parameterized as follows: *ppm* = 2, *peakwidth* = c(5,20), *prefilter* = c(1,15000000), *mzCenterFun* = “apex,” *integrate* = 1, *mzdiff* = -0.001, *fitgauss* = F, *sntresh* = 10. Once peak detection was complete, we trimmed the resulting polarity-specific data frames based on retention time and retained only those peaks detected between 1 and 21 min.

A major shortfall of employing LC-MS to perform “untargeted profile analysis,” as we did here, is the production of two independent but partially overlapping datasets resulting from ion polarity switching. While polarity switching is useful for detection of features that can only be detected via either positive or negative ionization, some features are detectable under both ionization modes, therefore resulting in two independent data sets containing a small subset of common features. Moreover,

571 interpretation of statistical results is challenging due to the
572 presence of parallel sets of analyses with common features
573 contributing to both. To alleviate these issues, we combined
574 positive and negative polarities using the *find.matches* function in
575 the *Hmisc* package (Harrell and Dupont, 2018). The *find.matches*
576 function allows one to identify which rows in a data matrix align
577 with those in a separate, identically formatted matrix by allowing
578 the user to define a tolerance level for the numerical columns
579 in each matrix. Thus, to determine our common features in the
580 positive and negative ionization datasets that result from LC-
581 MS, we created two matrices for positive and negative polarity,
582 containing three separate columns – the mass of each detected
583 peak, an assigned name for each peak, and retention time. To
584 ensure that corresponding features from each ionization mode
585 were capable of alignment, we subtracted 2.1046, roughly the
586 mass of two protons, from all masses in the positive polarity
587 dataset. For those features identified as common among both
588 ionization modes, we retained peak data from the polarity
589 exhibiting greatest mean intensity across all samples. We then
590 assigned new peak names to identify which peaks were present
591 in either positive or negative polarity vs. those that were found
592 in both. All output created using the *find.matches* function was
593 manually checked to ensure that all peaks identified as having a
594 match in one polarity, had their mate identified as a match in the
595 other.

596 We used permutational MANOVA (perMANOVA, Anderson,
597 2001) to compare PSM profiles between abiotic conditions.
598 When analyzing PSM profiles, differences were estimated using
599 Canberra dissimilarity matrices (Dixon et al., 2009). Analysis was
600 performed with the *adonis* function (from the *vegan* package,
601 Oksanen et al., 2015), which allowed us to account for our
602 blocked sampling design via the *strata* argument. Both differences
603 in the centroids among conditions or differences in multivariate
604 dispersion can lead to statistically significant results when
605 using perMANOVA. To determine whether differences among
606 centroids were contributing to perMANOVA results, we created
607 mean dissimilarity matrices using the *meandist* function and we
608 used the *betadisper* function to assess multivariate homogeneity
609 of variance (i.e., dispersion, Oksanen et al., 2015). We used
610 non-metric multidimensional scaling (NMDS, Kruskal, 1964) to
611 visualize differences in PSM profiles among conditions, which
612 we performed using the *metaMDS* function in the *vegan* package
613 (Oksanen et al., 2015). We set our dimensionality parameter (*k*)
614 to 2 and projected condition-specific effects onto NMDS plots
615 using the *ordiellipse* function to plot 95% confidence ellipses
616 based on standard error (Oksanen et al., 2015).

617 To evaluate treatment-induced changes to PSM diversity, we
618 calculated phytochemical richness based on the presence and
619 absence of individual compounds, then tested the main effect
620 of treatment on richness with block (experimental samples)
621 or site ID (observational samples) as our random effect using
622 linear mixed-effects models (*lme* function within the *nlme*
623 package, Pinheiro et al., 2015). To analyze phytochemical
624 turnover (i.e., the degree of overlap between the phytochemical
625 profiles of individual plants across and between conditions),
626 we created dissimilarity matrices based on binary datasets
627 representing the presence or absence of each feature using

Jaccard's Index. We evaluated condition-specific differences in
628 phytochemical turnover using perMANOVA via the *adonis*
629 function, and evaluated the influence of multivariate centroids
630 and homogeneity of variance on perMANOVA results as detailed
631 above (Anderson, 2001; Oksanen et al., 2015).
632

633 Weather data from CFC shows that ambient air temperature,
634 cumulative precipitation from 1 January to collection date, and
635 leaf surface temperature were not statistically different between
636 2012 and 2013 or between specific sample sets (2013 – closed
637 overstory, 2014 – open overstory). However, soil moisture and
638 soil temperature vary strongly between years and sample sets, and
639 differences between experimental and observational samples are
640 likely to be even greater. Thus, samples collected during different
641 years were analyzed independently of one another as individual
642 data sets.

643 For analytical and visualization purposes, the condition or set
644 of conditions assumed to impart the least amount of metabolic
645 change during each year was labeled as our reference group, to
646 which all other conditions were compared for that sample year.
647 For Year 1 (2013), we designated “ambient” as our reference
648 category, while samples grown under ambient temperature and
649 ambient precipitation were designated as our reference category
650 for Year 2 (2014). We designated samples collected from cold
651 region, low-light conditions as our reference category for Year
652 3 (2015). To help visualize how different abiotic conditions may
653 influence PSM production in different species, we calculated
654 the number of chemical features that increased and decreased
655 by $\geq 75\%$, relative to our reference category and created scaled
656 Venn Diagrams representing these relationships.

657 Finally, we used linear mixed-effects models to test the main
658 effect of abiotic condition on the relative abundance of our
659 example compounds, with sample block as our random effect
660 for experimental samples and plot ID as our random effect for
661 observational samples (*lme* function within the *nlme* package,
662 Pinheiro et al., 2015). These models tested whether combinations
663 of abiotic factors influence the abundance of our known example
664 compounds.
665

666 RESULTS

667 Temperature

668 The influence of temperature was both species and context
669 dependent. In closed overstory (Year 1), when compared to
670 ambient, warming-induced changes to the phytochemical
671 profile of balsam fir were not statistically significant, whereas
672 paper birch exhibited warming-induced shifts to phytochemical
673 profiles, thereby leading to distinct PSM profiles for the
674 warming treatment. Analysis of multivariate dispersion and
675 mean-dissimilarity matrices both suggest that differences in
676 paper birch were due to temperature-induced changes in the
677 centroid rather than dispersion (Table 1). NMDS plots reveal
678 minor overlap between temperature conditions in paper birch,
679 and balsam fir grown under moderate and high-temperatures
680 show strong overlap with plants grown in ambient temperatures
681 but minor overlap with each other (Figure 2). Warming had
682 no effect on phytochemical richness in either species but did
683
684

TABLE 1 | Summary of results for B4WarmED samples used to assess the influences of temperature and drought on PSM profiles and phytochemical diversity.

Year	Species	Stress condition	n	Features	PSM profile				Phytochemical diversity					
					perMANOVA		Dispersion		Centroid		LME _{richness}		perMANOVA _{turnover}	
					F	P	F	P	Δ	Δ _{richness}	P	P	F	P
2013	Balsam fir	Ambient ^a	12	1903	1.223	0.073	0.103	0.576	0.567	na	na	1.206	0.072	0.142
		Mod. temp.	13	1856						-25.800	-47	0.154		
		High temp.	9	1873						-68.500	-30	0.321		
		Ambient ^a	11	1669	1.382	0.090	0.013*	0.765	0.470	na	na	1.444	0.093	0.019*
		Mod. temp.	12	1722						55.700	53	0.201		
2014	Balsam fir	High temp.	8	1700						17.700	31	0.526		
		Ambient ^a	5	1937	1.016	0.105	0.428	0.346	0.810	na	na	1.076	0.110	0.308
		Temp.	11	2017						196.000	80	0.222		
		Drought	5	2012						121.000	75	0.308		
		Temp. + drought	9	1992						118.000	55	0.308		
	Red maple	Ambient ^a	5	1968	1.070	0.100	0.303	1.520	0.210	na	na	1.076	0.100	0.320
		Temp.	11	2002						29.300	34	0.800		
		Drought	4	1998						97.600	30	0.857		
		Temp. + drought	13	1845						-251.300	-123	0.344		
		Ambient ^a	6	1948	1.149	0.097	0.147	1.233	0.307	na	na	1.210	0.102	0.134
	Paper birch	Temp.	12	2014						32.000	66	0.232		
		Drought	7	1949						-112.000	1	0.973		
		Temp. + drought	11	2036						98.000	88	0.122		
		Ambient ^a	4	2287	0.689	0.103	0.960	0.061	0.980	na	na	0.622	0.094	0.980
		Temp.	6	2282						17.000	-5	0.961		
	Trembling aspen	Drought	5	2241						-44.000	-46	0.646		
		Temp. + drought	7	2282						16.000	-5	0.957		

For samples collected during 2013, "mod. temp." includes all samples collected from plots warmed to ambient + 1.7°C, while "high temp." includes all samples collected from plots warmed to ambient + 3.4°C. For a given stress condition, the mean number of chemical features identified within a species is listed under "features." "Dispersion" represents the results of our multivariate homogeneity of variance test, while "centroid" represents the mean difference in dissimilarity matrices relative to our reference group (*). A larger Δ value indicates greater distance from the reference group than those with a smaller Δ. All statistical analyses were tested against α = 0.05, and statistically significant results are italicized and identified with an asterisk (*). ^aReference group or baseline condition for the given sample year to which all other treatments within species were compared. na indicates not applicable.

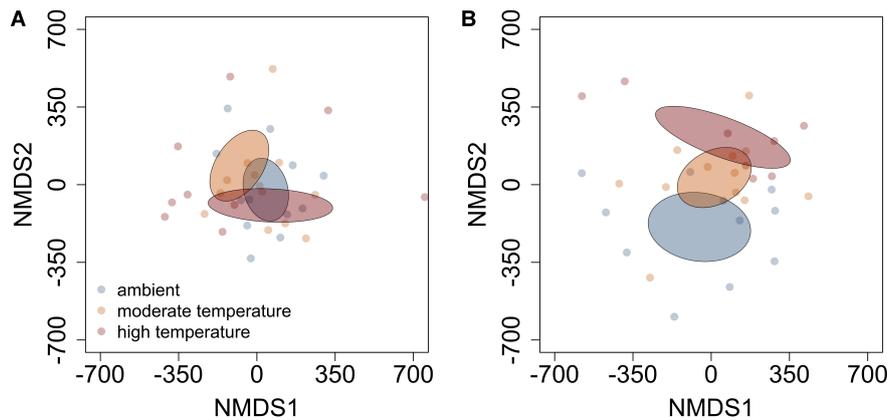


FIGURE 2 | Non-metric multidimensional scaling (NMDS) plots detailing the influence of moderate and high-temperature on PSM profiles of **(A)** balsam fir and **(B)** paper birch in closed overstory. Ellipses represent 95% confidence intervals, based on standard error. In balsam fir, both warming treatments exhibit less overlap with each other than with ambient. In paper birch, different temperatures lead to distinct profiles when compared to each other and ambient.

influence phytochemical turnover in paper birch (Table 1). In open overstory (Year 2), warming had no influence on PSM profiles or PSM diversity (i.e., richness or turnover), regardless of species (Table 1). NMDS plots support these findings in that there is no discernible relationship between temperature and PSM profiles, regardless of species (Figure 3). In observational samples collected throughout northeast Minnesota (Year 3), temperature on its own had no influence on plant PSM profiles or phytochemical richness values. However, phytochemical turnover was significantly different in plants from different temperature regions in paper birch (perMANOVA, $F = 5.912$, $r^2 = 0.179$, $P = 0.0003$) and trembling aspen (perMANOVA, $F = 3.322$, $r^2 = 0.156$, $P = 0.0012$). NMDS plots suggest that each species responds differently to combinations of temperature and light (i.e., canopy; Figure 4). Balsam fir produces distinct PSM profiles as a function of ambient light conditions (i.e., open vs. closed canopy), but only within the cool region, while paper birch and trembling aspen appear to have distinct PSM profiles for each combination of conditions. Conversely, beaked hazel exhibits no discernible pattern across different conditions.

Venn diagrams created to help visualize the influence of different abiotic conditions for Year 1 samples suggest that the high-temperature (+3.4°C) treatment induced a greater response from both balsam fir and paper birch than the moderate-temperature (+1.7°C) treatment. Specifically, the high-temperature treatment led to more features that either increased or decreased in relative abundance by 75% or more when compared to ambient or moderate-temperature treatments (Table 2 and Supplementary Figures S4–S6).

Interactive Effects of Different Abiotic Conditions

In our Year 2 samples, the combination of drought and elevated temperature had no influence on PSM profiles or any aspect of phytochemical diversity, regardless of species (Table 1). These results were supported by NMDS plots (Figure 3). Additionally, Venn diagrams suggest large-magnitude increases or decreases in

relative abundance of PSMs did not follow an obvious pattern that could be attributed to different conditions. There appears to be a high degree of overlap across conditions in those compounds that exhibit increases in relative abundance of $\geq 75\%$, while less overlap occurs among compounds exhibiting large declines in relative abundance. Furthermore, the influence of drought on the decline of relative abundance by $\geq 75\%$ appears to be more distinct than that of either warming or warming and drought together (Table 2 and Supplementary Figures S4–S6).

In observational samples from throughout northeast Minnesota (Year 3), when evaluating the effects of high temperature and light combined, balsam fir appears to create unique PSM profiles in response to different light conditions (i.e., open vs. closed canopy), but only within the cool region, while paper birch and trembling aspen appear to have distinct PSM profiles for each condition. Beaked hazel exhibits no discernible pattern (Figure 4). Phytochemical richness did not vary as a function of light conditions or temperature region. However, phytochemical turnover in balsam fir was significantly influenced by conditions of high light (i.e., open canopy; Table 3). When analyzing the interactive effects of light conditions and temperature region, all species exhibited significant differences in their PSM profile (Table 3), with only trembling aspen exhibiting significant differences in multivariate dispersion as a function of the combination of light condition and temperature region (Table 3). Although phytochemical richness was not influenced by the combined effects of temperature region and light conditions, phytochemical turnover was influenced in paper birch and trembling aspen and a marginal, non-significant trend was present in beaked hazel (Table 3).

Patterns in Venn diagrams detailing the influences of different conditions during Year 2 are difficult to discern, as different plant species appeared to respond to varying conditions in different ways (Table 2 and Supplementary Figure S5). Drought led to more features increasing by $\geq 75\%$ in balsam fir and paper birch, while elevated temperature led to the large-magnitude increase of more features in trembling aspen (Table 2 and

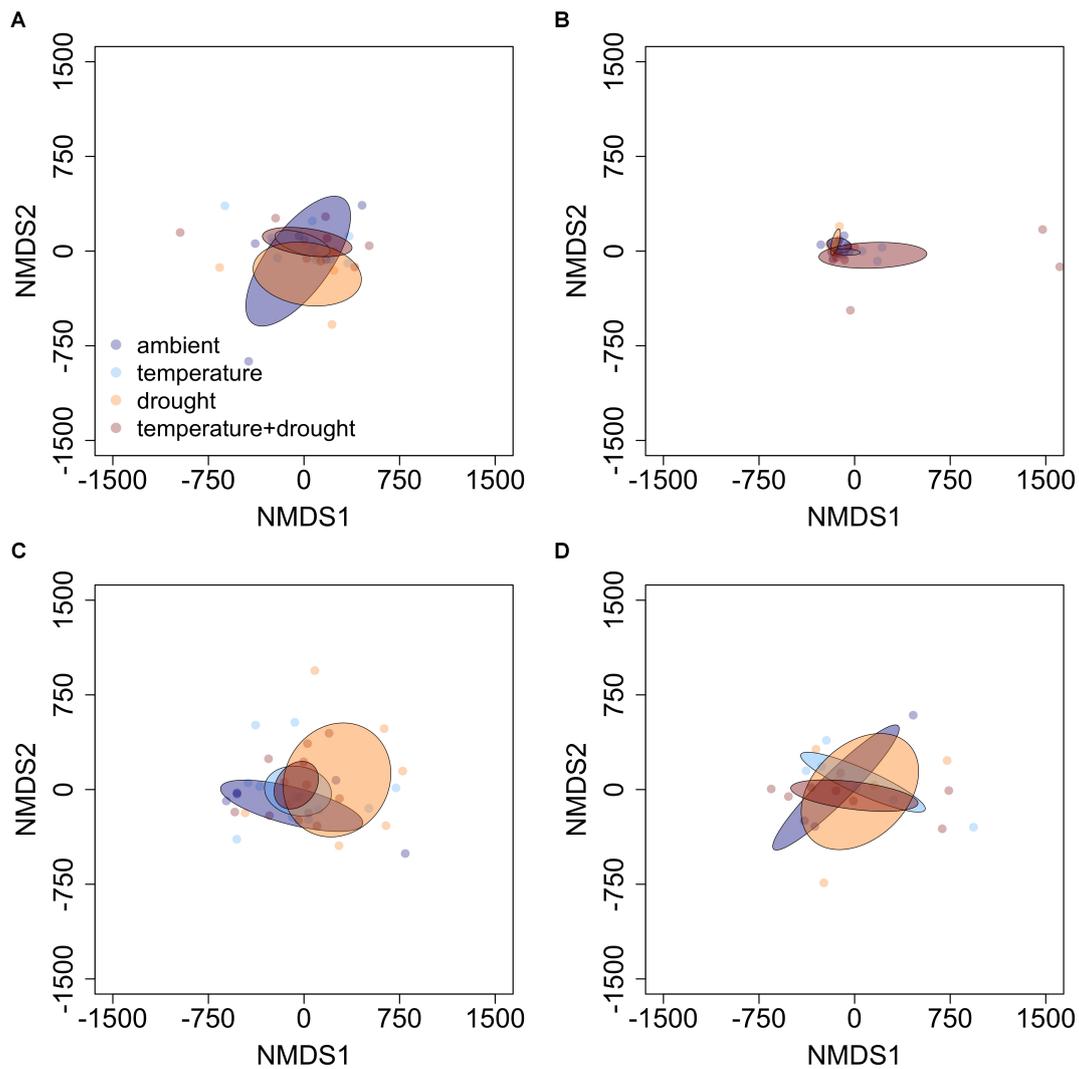


FIGURE 3 | Non-metric multidimensional scaling (NMDS) plots detailing the influence of elevated temperature and drought on PSM profiles of **(A)** balsam fir, **(B)** red maple, **(C)** paper birch, and **(D)** trembling aspen in open overstory. Ellipses represent 95% confidence intervals, based on standard error. There appears to be no discernible pattern between sets of abiotic factors and PSM profiles, regardless of species.

Supplementary Figure S5). In red maple, the combination of drought and elevated temperature had the greatest influence on large-magnitude increases in relative abundance (Table 2 and Supplementary Figure S5). The combination of drought and warming led to more large-magnitude declines in relative abundance in balsam fir and paper birch, while drought had a greater impact on red maple and trembling aspen (Table 2 and Supplementary Figure S5). In observational samples (Year 3), the combination of high-light conditions and warmer temperatures led to more large-magnitude shifts in relative abundance (i.e., increasing and decreasing by 75% or more), regardless of species (Table 2 and Supplementary Figure S6).

Example Compounds

In closed-overstory conditions (Year 1), warming resulted in significant declines in both catechin and terpene acid in

paper birch but had no influence on either compound in balsam fir (Figure 5 and Supplementary Table S3). In high-light conditions (Year 2), neither of the compounds in either species exhibited a significant, condition-specific change in relative abundance. However, terpene acid in paper birch was completely absent from all samples collected from high-light plots (Figure 6 and Supplementary Table S3). In observational samples from throughout northeast Minnesota (Year 3), neither compound in balsam fir exhibited significant changes in relative abundance due to light conditions, temperature region, or their interaction. In paper birch, however, the interactive effects of high-light conditions and warmer-temperatures resulted in a more than 250% increase in the relative abundance of catechin, while terpene acid exhibited no response, regardless of treatment (Figure 7 and Supplementary Table S3).

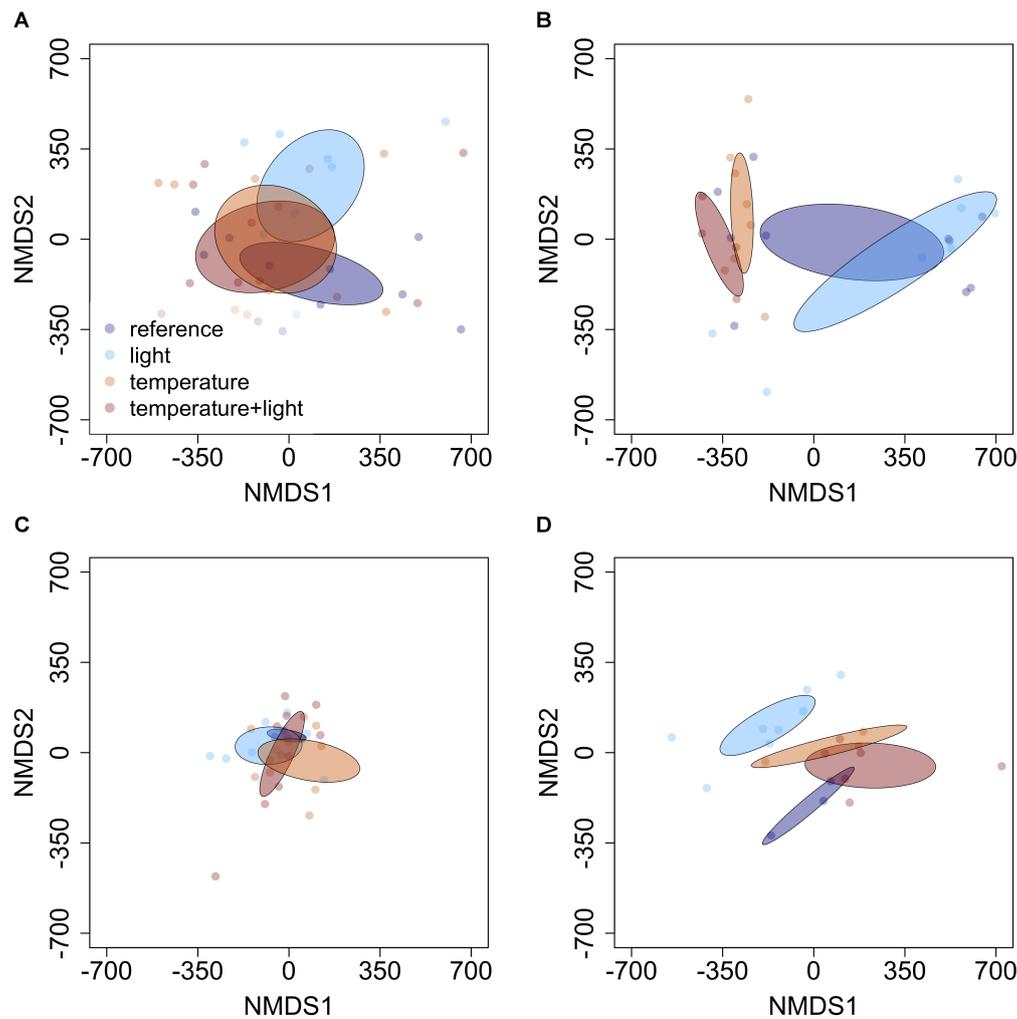


FIGURE 4 | Non-metric multidimensional scaling (NMDS) plots detailing the influence of varying light and temperature conditions on PSM profiles of **(A)** balsam fir, **(B)** paper birch, **(C)** beaked hazel, and **(D)** trembling aspen. Ellipses represent 95% confidence intervals, based on standard error. Each species appears to respond to different abiotic conditions in a unique manner. Balsam fir appears to create unique PSM profiles in high-light conditions when compared to our reference group (closed canopy, low temperature), while paper birch and trembling aspen appear to have distinct PSM profiles for each set of conditions. Beaked hazel exhibits no discernible pattern.

DISCUSSION

Our study is among the first to explicitly show that combinations of abiotic drivers (often potential stressors) in forest plants can lead to broad phytochemical responses that are distinct from those that result from single abiotic factors and that different species of woody plants respond to complex sets of conditions in variable ways. In our experimental samples, warming under closed canopy led to distinct PSM profiles in paper birch but not balsam fir, with paper birch exhibiting increased phytochemical turnover. Warming under open canopy had no influence on PSM profiles or any aspect of phytochemical diversity. In our observational samples collected across northeast Minnesota, warmer temperatures had no influence on PSM profiles but did lead to significant phytochemical turnover in paper birch and trembling aspen. When elevated temperature was combined with

drought in Year 2 of our experimental samples, we found no influence on PSM profiles or phytochemical diversity. However, temperature variation combined with high-light conditions in our observational samples resulted in condition-specific profiles for all species and led to significant phytochemical turnover in all but beaked hazel. In general, our results indicate that the phytochemical response of plants to varying combinations of abiotic factors cannot be directly extrapolated from the response of plants to individual factors. Perhaps more importantly, our results provide evidence that heterogeneity in the abiotic environment influences secondary metabolism in woody plants via a range of complex and highly variable responses.

Few studies to date have explicitly studied the influences of heterogeneity in the abiotic environment on phytochemical diversity, and specifically, phytochemical turnover. However, it has been hypothesized that variability in which compounds

TABLE 2 | Number of chemical features that increase and decrease in relative abundance by $\geq 75\%$ as a function the dominant stress condition.

Year	Species	Increase by $\geq 75\%$		Decrease by $\geq 75\%$	
		Stress condition	Number affected	Stress condition	Number affected
2013	Balsam fir	High Temperature	6	High Temperature	21
	Paper birch	High Temperature	28	High Temperature	38
2014	Balsam fir	Drought	43	Temperature + Drought	35
	Paper birch	Drought	98	Temperature + Drought	31
	Red maple	Temperature + Drought	36	Drought	66
2015	Trembling aspen	Temperature	79	Drought	37
	Balsam fir	Temperature + Light	26	Light	111
	Beaked hazel	Temperature + Light	155	Temperature + Light	56
	Paper birch	Temperature + Light	126	Light	278
	Trembling aspen	Temperature + Light	280	Light	162

In most scenarios, the stress condition that led to large-scale increases in relative abundance was different than that which led to large-scale decreases. "Number affected" displays the number of chemical features that either increased or decreased by $\geq 75\%$ for the given species and stress condition.

are either present or absent may be an adaptation for variable environments, thereby decreasing vulnerability of plants to a range of potential stress conditions, including herbivory (Laitinen et al., 2000; Cheng et al., 2011). Here, we found that in some plants species, different combinations of abiotic factors can affect which compounds are either present or absent, thus leading to phytochemical turnover. For example, compounds that are absent in one set of conditions may become present within a slightly different set of conditions, or vice versa. The potential for this to occur was apparent when our example terpene acid decreased in paper birch plants subjected to experimentally elevated temperature in closed canopy but went completely undetected in plants subjected to experimental warming and drought in open canopy and exhibited no change at all in our observational samples from throughout northeast Minnesota. Suppression of individual compounds due to varying stress conditions has been observed in other studies as well. For instance, proline, which is thought to play an important role in protection from drought, is severely suppressed when plants are simultaneously subjected to drought and high temperatures (Rizhsky et al., 2004). While individual compounds can play an important role in the survival of plants subjected to a range of biotic and abiotic conditions, a plant's phytochemical profile imparts a metabolic framework that can determine the biological role and strength of individual compounds (Dyer et al., 2003; Richards et al., 2010; Gershenson et al., 2012; Jamieson et al., 2015). Here, we show that individual compounds as well as the phytochemical context within which they operate can both be altered by variations in the abiotic environment.

Plants produce thousands of individual compounds, and variations in the relative abundance of many of these compounds can have a wide-range of effects on the biotic interactions plants have with other organisms. Catechin, which is a phenol-based precursor to proanthocyanidins (i.e., condensed tannins), is widely considered an antiherbivore defensive compound (Tahvanainen et al., 1985; Berg, 2003; Stolter et al., 2005) and can have a significant, negative impact on the development of forest pests (Roitto et al., 2009). Catechin also has antimicrobial

and allelopathic effects, and plants with decreased catechin production may be at a competitive disadvantage for nutrients within the soil as it can inhibit the growth and germination of neighboring plants (Veluri et al., 2004; Inderjit et al., 2008). Terpene acids, including diterpene resin acids, are considered strong antifeedants (Ikeda et al., 1977), and the ingestion of forage with elevated concentrations of diterpenoids can result in slower development times and significantly higher mortality in herbivorous larvae (Larsson et al., 1986). Here, we show that different compounds have individualized responses based on the micro-environmental conditions that are present.

In balsam fir, warming alone led to consistent, albeit non-significant declines in the mean relative abundances of both resin acids. When high temperatures were combined with other abiotic factors (i.e., drought and light), resin acid 1 exhibited consistent but non-significant increases, while resin acid 2 was more variable, exhibiting no consistent trend. In paper birch, both example compounds exhibited significant changes in relative abundance as a function of different factors. While elevated temperature alone led to significant declines in catechin, the combination of elevated temperature and high light led to a more than 250% increase in relative abundance. Our example terpene acid declined with warming and was undetectable when we tried to assess the effects of drought. This particular scenario provides an example of how individual compounds may "wink in or out" due to variation in the abiotic environment.

Numerous studies have reported that high-temperature and drought interact to alter PSM production in plants (Craufurd and Peacock, 1993; Savin and Nicolas, 1996; Jiang and Huang, 2001; Rizhsky et al., 2002, 2004). Thus, we were surprised to find no interaction between drought and warming in our study. It is important to note, however, that the extremes of those treatments employed by other studies are typically greater than what we test here, sometimes increasing temperature to more than 40°C (Rizhsky et al., 2002) and withholding water altogether for extended periods (Jiang and Huang, 2001). In our study, mean soil moisture was lower during 2014 than 2013, with mean soil temperatures being higher (unpublished

TABLE 3 | Summary of results for observational samples used to assess the influences of temperature region and overstory on PSM profiles and phytochemical diversity.

Year	Species	Stress condition	n	Features	PSM profile				Phytochemical diversity																		
					perMANOVA	Dispersion	Centroid	LME _{richness}	perMANOVA _{turnover}	F	r ²	P															
2015	Balsam fir	Reference ^a	10	1371	F	1.579	r ²	0.119	P	0.024*	F	0.334	P	0.807	Δ	na	Δ _{richness}	na	P	na	F	2.152	r ²	0.156	P	0.004*	
		Light	8	1287																							
	Paper birch	Temp. + light	10	1373																							
		Reference ^a	11	1361																							
	Beaked hazel	Light	10	1185																							
		Temp.	7	1168																							
	Trembling aspen	Temp. + light	8	1205																							
		Reference ^a	4	1223																							
		Light	3	1338																							
		Temp.	8	1220																							
		Temp. + light	12	1194																							
		Reference ^a	10	1252																							
		Light	3	1509																							
		Temp.	8	1466																							
		Temp. + light	3	1531																							
		Reference ^a	6	1558																							

For a given condition, the mean number of chemical features identified within a species is listed under "features." "Dispersion" represents the results of our multivariate homogeneity of variance test, while "centroid" represents the mean difference in dissimilarity matrices relative to our reference group (*). A larger Δ value indicates greater distance from the reference group than those with a smaller Δ. All statistical analyses were tested against α = 0.05, and statistically significant results are italicized and identified with an asterisk (*). ^aReference group or baseline condition (i.e., lower temperatures, low light) to which all other treatments were compared. na indicates not applicable.

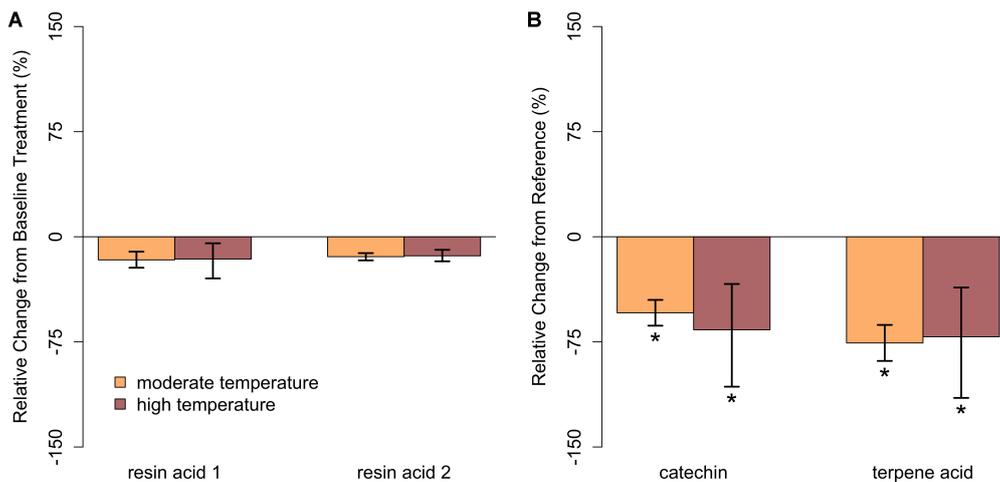


FIGURE 5 | Relative change in abundance (%) for specific PSM compounds when compared to our reference treatment for Year 1 (ambient temperature) for (A) balsam fir and (B) paper birch in closed overstory. Neither resin acid in balsam fir was influenced by warming. In paper birch, both catechin and terpene acid declined with warming relative to ambient. Error bars represent the 95% boot-strapped confidence intervals and relative abundances significantly different than those found in the baseline treatment are identified by an asterisk (*).

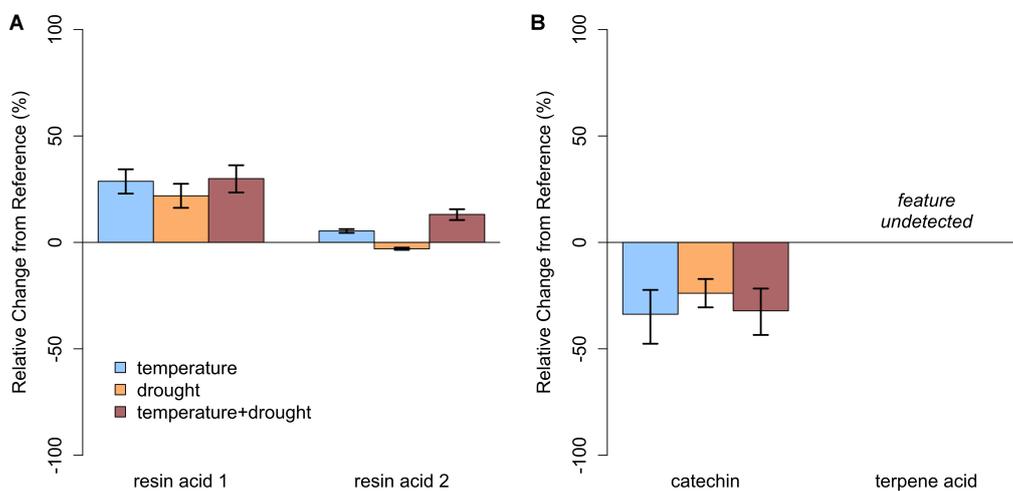


FIGURE 6 | Relative change in abundance (%) for specific PSM compounds when compared to our baseline treatment for Year 2 (ambient temperature, ambient precipitation) for (A) balsam fir and (B) paper birch in open overstory. Neither resin acid in balsam fir was influenced by warming. In paper birch, relative abundance of catechin was not influenced by temperature; however, terpene acid was undetected. Error bars represent the 95% boot-strapped confidence intervals.

data). Surprisingly, air temperature and leaf-tissue surface temperature during late spring/early summer (May 1 to July 15) were indistinguishable between samples years and plot types (2013 closed canopy vs. 2014 open canopy), and cumulative precipitation during the first half of each year (January 1 to July 15) was also indistinguishable (unpublished results). Combinations of abiotic factors can have one dominant factor that defines the phytochemical response of affected plants, and drought, when present, may dominate the influence of combinations of abiotic factors. Considering this, our inability to identify any treatment-specific influence on PSM profiles or phytochemical diversity may be due to low soil moisture during 2014. If plants from which samples were collected from

in 2014 were experiencing some level of drought stress due to low soil moisture, this signal may have preempted any potential phytochemical response that might have occurred due to treatment.

When considering the influence of abiotic conditions on large-scale shifts in relative abundance (increases or decreases $\geq 75\%$ relative to our reference group), greater increases in temperature ($+3.4^{\circ}\text{C}$) appeared to have a greater influence than moderate increases ($+1.7^{\circ}\text{C}$). When present, drought, either alone or in combination with elevated temperature, dominated all but one of the large-scale shifts we assessed (Year 2), and in our observational samples, high-light conditions, either alone or in combination with elevated temperature, dominated all

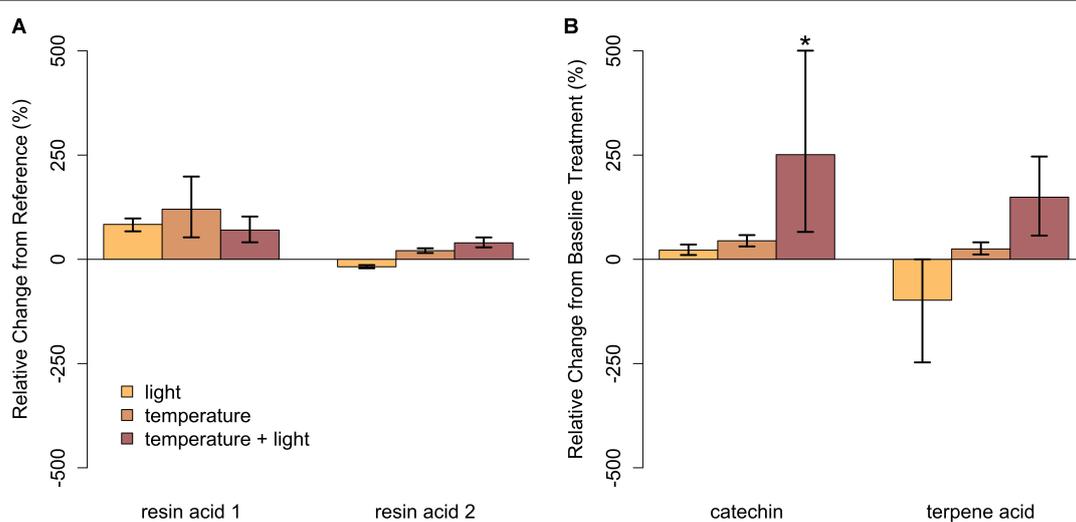


FIGURE 7 | Relative change in abundance (%) for specific PSM compounds when compared to our baseline treatment for Year 3 (cold region, closed overstory) for (A) balsam fir and (B) paper birch. Neither resin acid in balsam fir was influenced by warming. In paper birch, relative abundance of catechin was only influenced by the combination of light and high temperatures, increasing by more than 250%. Terpene acid was unaffected, regardless of stress condition. Error bars represent the 95% boot-strapped confidence intervals and relative abundances significantly different than those found in the reference condition are identified by an asterisk (*).

of the large-scale shifts we assessed in which it was present (Year 3). As noted above, numerous studies have reported that drought has a defining impact on plants' phytochemical profiles, even when in combination with other abiotic drivers, such as elevated temperature and high light. Moreover, in our Year 1 samples, elevated temperature led to both large-scale increases and large-scale decreases in relative abundance. However, the number of compounds exhibiting these shifts was substantially smaller when compared to the number of compounds influenced by the abiotic conditions evaluated in either Year 2 of our experimental samples or our observational samples (Year 3). Outside of Year 1, during which we tested only the effects of elevated temperature, it was rare when the same abiotic condition simultaneously dominated both large-scale increases and large-scale decreases in relative abundance, suggesting that different combinations of abiotic factors may influence upregulation and downregulation of different compounds.

Changes in the abundance and diversity of secondary metabolites within a plant's phytochemical profile may alter biotic interactions, potentially leading to broad-scale ecological change. For example, while some herbivores respond negatively to forage with a higher diversity of PSMs, others appear to target these plants in an effort to alleviate costs associated with external stressors via their pharmacological benefits (Forbey and Hunter, 2012). Additionally, numerous studies have reported that phytochemical diversity within a plant community is positively correlated with community diversity across multiple trophic levels (Jones and Lawton, 1991; Richards et al., 2015), influencing invertebrate predators and parasitoids, and potentially extending to vertebrate predators as well (Dicke et al., 2012).

While the consequences of different abiotic conditions on phytochemical diversity remain unpredictable, our results

demonstrate that the phytochemical response of plants to combinations of abiotic factors cannot be extrapolated from that of individual factors. For instance, while warming alone may have a very specific influence on some compounds, when in combination with additional abiotic factors such as drought and light, warming may lead to highly variable and unpredictable response (Mittler, 2006), making it increasingly difficult to predict the performance of woody plants in a changing environment. Regardless, previous research suggests that changes in phytochemical production induced by variability in abiotic conditions can influence both tree resistance and pest performance traits (Jamieson et al., 2015), potentially altering the frequency and intensity of insect outbreaks (Schwartzberg et al., 2014). Elevated temperatures by themselves have been shown to reduce the competitive abilities of more southern boreal tree species when compared to co-occurring species adapted to warmer climates (Reich et al., 2015). Climate-induced changes to phytochemistry may lead to shifts in the competitive landscapes for cold-adapted trees and shrubs, potentially altering their ability to compete for resources and defend against pests and pathogens in novel climatic conditions. However, because individual compounds and the metabolic profiles of which they are a part are differentially influenced by abiotic factors and combinations of these factors, predicting how forest plants will respond to novel environmental conditions will be challenging.

The majority of biotic interactions between plants and other organisms are chemically mediated, and recent climate change has challenged our understanding of the mechanisms underlying these interactions. The primary objective of this study was to determine how warming influences plant production of secondary metabolites and how combinations of additional abiotic factors may modulate this effect.

1597 Here, we show that heterogeneity in a range of abiotic factors
 1598 broadly influence secondary chemistry in plants thereby leading
 1599 to condition-specific phytochemical profiles. If our results
 1600 are typical of plant responses, abiotically induced changes to
 1601 secondary chemistry in woody plants could influence their
 1602 rate of range expansion or contraction under novel climate
 1603 scenarios. Additionally, our results contribute to current efforts
 1604 to understand how continued warming will influence plants and
 1605 the biotic interactions that serve as the foundation for a wide
 1606 range of ecosystem processes. In the future, studies monitoring
 1607 physiological changes in conjunction with global shifts in PSM
 1608 profiles would provide insights into mechanisms underlying
 1609 biotic interactions mediated by the local environment. As spatial
 1610 and temporal patterns in the global abiotic environment continue
 1611 to shift, it is imperative that we continue to learn as much as
 1612 we can about the phytochemical response of plants to these
 1613 changes.

1614 AUTHOR CONTRIBUTIONS

1615
 1616 JB, SB, AH, RaM, ReM, and JF formulated the study idea and
 1617 developed the study methods while PR and ReM established
 1618 the experimental study sites critical for the execution of this
 1619 study. JB performed all the sample collection, while JB and
 1620 SB performed the analytical chemistry and pre-statistical data
 1621 processing. JB, RaM, and JF statistically analyzed the data. JB
 1622 and SB wrote the initial draft of manuscript. All the authors
 1623 contributed to the manuscript revisions and approved the final
 1624 manuscript.

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SUPPLEMENTARY MATERIAL

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Habitat functional response mitigates reduced foraging opportunity: implications for animal fitness and space use

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Abstract

Context Animals selectively use landscapes to meet their energetic needs, and trade-offs in habitat use may depend on availability and environmental conditions. For example, habitat selection at high temperatures may favor thermal cover at the cost of reduced foraging efficiency under consistently warm conditions.

Objective Our objective was to examine habitat selection and space use in distinct populations of

moose (*Alces alces*). Hypothesizing that endotherm fitness is constrained by heat dissipation efficiency, we predicted that southerly populations would exhibit greater selection for thermal cover and reduced selection for foraging habitat.

Methods We estimated individual step selection functions with shrinkage for 134 adult female moose in Minnesota, USA, and 64 in Ontario, Canada, to assess habitat selection with variation in temperature, time of day, and habitat availability. We averaged model coefficients within each site to quantify selection strength for habitats differing in forage availability and thermal cover.

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Results Moose in Ontario favored deciduous and mixedwood forest, indicating selection for foraging habitat across both diel and temperature. Habitat selection patterns of moose in Minnesota were more dynamic and indicated time- and temperature-dependent trade-offs between use of foraging habitat and thermal cover.

Conclusions We detected a scale-dependent functional response in habitat selection driven by the trade-off between selection for foraging habitat and thermal cover. Landscape composition and internal state interact to produce complex patterns of space use, and animals exposed to increasingly high temperatures may mitigate fitness losses from reduced foraging efficiency by increasing selection for foraging habitat in sub-prime foraging landscapes.

Keywords Alces · GPS · Habitat selection · Heat stress · Lasso · Model selection · Moose · Movement

Introduction

Animal fitness is constrained by energetic considerations—that is, organisms must take in enough energy to maintain internal conditions, grow, and reproduce. This observation implies an economy of energy central to animal ecology, where the rate of energy output is balanced by the rate of energy input. In this supply-expenditure framework, ecologists frequently consider the supply component in the form of resource acquisition, processing, and energy storage. This has led to the idea that metabolic rates scale with body size and temperature, and that energy and resources are fundamentally and functionally relatable (i.e., the metabolic theory of ecology; Brown et al. 2004). The underlying assumption of such studies is that energy supply is the primary constraint on individual fitness; organisms attempt to meet an energy budget wherein they must acquire enough resources (or have enough in storage) to maintain homeostatic conditions while still producing and supporting offspring. Other studies have highlighted the importance of energy expenditure in balancing the metabolic equation. For example, an organism's maximum energetic expenditure may be limited by the combined metabolic rates of its component tissues (Hammond and Diamond 1997) or by its ability to dissipate heat (Speakman and Król

2010), and expenditure may sometimes supersede supply considerations, particularly under energy rich conditions. These two perspectives differ in which side of the energy equation is given priority—supply or expenditure—but both extol the importance of different components of a complex and dynamic system of energetic trade-offs.

If energy budget is a primary driver of animal fitness, it naturally follows that animals select habitat based on energetic considerations. Much research on energy acquisition has centered on foraging efficiency (e.g., Charnov 1976; Ritchie 1990; Illius et al. 1995), and how foraging considerations influence selective use of associated landscapes (e.g., Fryxell et al. 2008; Owen-Smith et al. 2010; Mitchell and Powell 2012). Comparatively little research has focused on energetic expenditure as a driver of space use and habitat selection and how it may influence fitness, particularly in endotherms (but see Aublet et al. 2009; Speakman and Król 2010; Van Beest et al. 2012). From this perspective, animals may exhibit preferences for habitats that reduce expenditure, for example by facilitating movement (Avgar et al. 2013) or by providing thermal cover (Van Beest et al. 2012; Street et al. 2015). Preference for habitats providing foraging opportunities or thermal cover should vary across the diurnal cycle and is likely dependent on animal state (e.g., hunger, thermal stress) and environmental context (Fryxell et al. 2008; Avgar et al. 2013). That environmental and landscape conditions are not constant over space can further alter both the supply and expenditure components of the energy equation, often simultaneously, thus a full examination of the relative importance of energy supply versus expenditure requires data across broad spatiotemporal extents and bioclimatic gradients to capture variation in habitat use across a variety of conditions and contexts.

Our objective was to investigate the influence of temperature and time of day on patterns of habitat selection and movement in an endothermic animal, the moose (*Alces alces*). Moose are generalist browsers that exhibit a strong preference for deciduous vegetation types (Belovsky 1981). They are also cold-adapted and exhibit physiological responses associated with heat stress at temperatures as low as 14 °C in the summer and −5 °C in the winter (Renecker and Hudson 1986). As such, the interaction between deciduous forage availability and temperature is often considered a primary driver of the southern limit of

moose population ranges. For example, Street et al. (2015) described changes in habitat selection by moose toward habitats providing thermal cover with increasing temperature at mid-day, suggesting that temperature regulation plays a role in moose behavior and space use at temperatures approaching moose thermal optima. However, this study was limited to a single population of moose and to locations at mid-day only. Such limitation is acceptable to the extent that habitat selection may be mediated by individual internal state (e.g., temperature) but is insufficient to fully characterize the relationship between temperature and behavior if that relationship is not consistent across space or time of day (Avgar et al. 2013). Alternatively, moose populations may be regulated at the southern extent of their range by factors including parasite loads (Murray et al. 2006) and predation (Mech and Fieberg 2014). Evaluating the potential for temperature to influence moose space use across landscapes would provide additional information about the realized niche of this species while simultaneously advancing our understanding of the interplay between biotic and abiotic conditions in shaping patterns of animal space use.

We estimated models of habitat selection and movement of individual moose across two study sites (northern Minnesota, USA, and northwest Ontario, Canada) representing ecologically distinct populations occupying landscapes of different composition. Given the hypothesis that endotherm fitness is constrained by efficiency of heat dissipation (Speakman and Król 2010), we predicted that (1) selection for land cover types by moose would vary with temperature and time of day, consistent with prior observations; and (2) moose in landscapes characterized by a higher abundance of thermal cover would select less strongly for these habitat types than moose in landscapes with limited shelter (or, more generally, that estimated selection for different landscape components is unique to a particular combination of habitat availability and environmental conditions).

Methods

Study area

This comparative study was conducted at two sites: one in northeast Minnesota at 47°50'N, 92°8'W; and

the other in northwest Ontario at 49°15'N, 92°45'W (Fig. 1).

The southern site is located in Minnesota's Northern Superior Uplands forest region northwest of Lake Superior (Hanson and Hargrave 1996). The northern site is located northwest of Ontario's Quetico Provincial Park on the Quetico-Great Lakes-St. Lawrence/boreal forest boundary (Rowe 1972). Both sites are a matrix of forested stands and wetlands (e.g., bogs, lakes). Dominant tree species are consistent between sites and include white spruce (*Picea glauca*), black spruce (*Picea mariana*), and jack pine (*Pinus banksiana*). Balsam fir (*Abies balsamea*), white birch (*Betula papyrifera*), and trembling aspen (*Populus tremuloides*) are interspersed throughout both sites. Both sites are primarily disturbed by forest fire and are subject to timber harvest. Moose co-occur throughout both study sites with white-tailed deer (*Odocoileus virginianus*) and are primarily preyed upon by gray wolves (*Canis lupus*) and black bears (*Ursus americanus*).

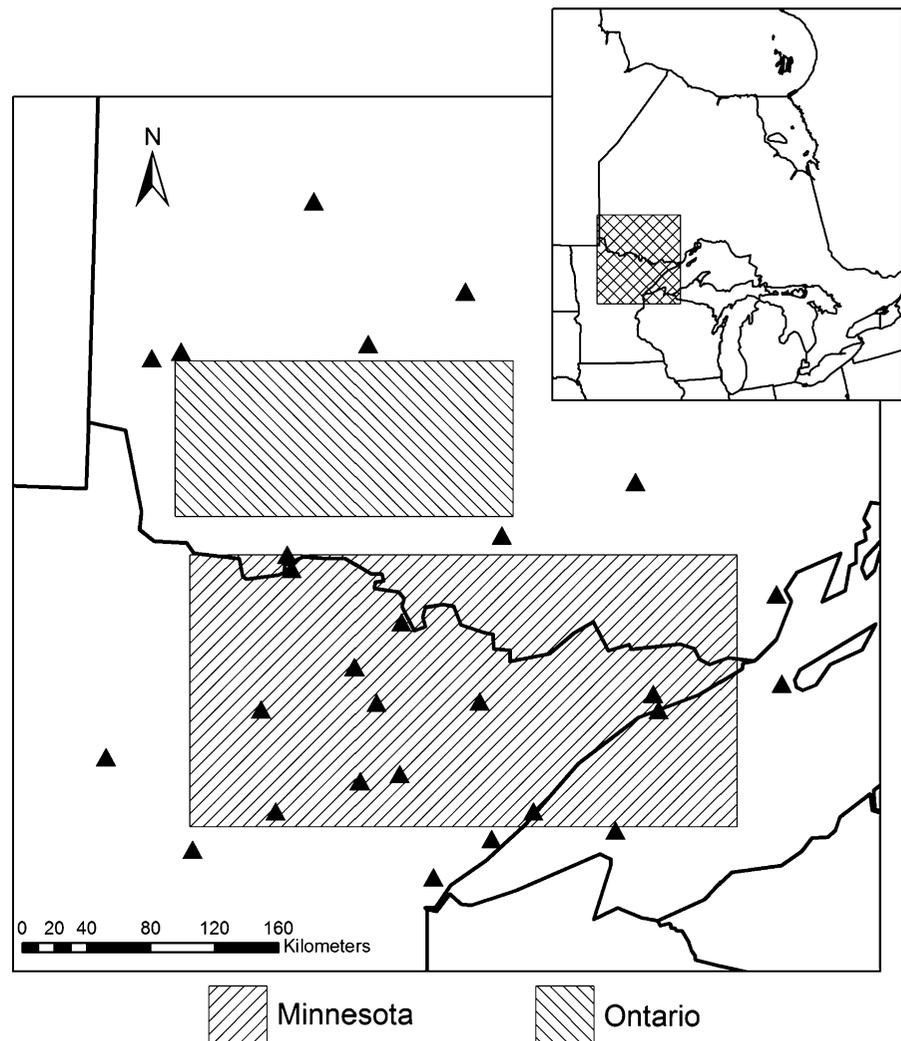
Data

In Minnesota throughout January and February from 2010 to 2015, technicians captured 170 adult female moose by netgunning or aerial darting from a helicopter using carfentanil (4.5 or 6.0 mg) or thifentanal (16 mg) and xylazine (100 or 150 mg) as immobilizing agents, and naltrexone (245–575 mg) and tolazoline (400 mg) as reversal agents. Moose were fitted with Iridium Global Positioning System (GPS) radiocollars (VECTRONIC Aerospace GmbH, Berlin, Germany; and Sirtrack Ltd., Hawkes Bay, New Zealand). Animal handling followed American Society of Mammalogists wild animal care guidelines (Sikes, Gannon & the Animal Care and Use Committee of the American Society of Mammalogists 2011). Collars recorded animal locations at 4.25, 2, and 1.065-h intervals, depending on region and study schedules. We subsampled data collected at higher sampling rates to achieve a consistent 4.25-h fix rate ± 0.25 h. See Carstensen et al. (2014) for more details.

In Ontario from 1995 to 2001, technicians captured 122 adult female moose by netgunning from a helicopter throughout the northern study site and deployed NAVSTAR GPS radiocollars (LOTEK Engineering Inc., Newmarket, Ontario) on each.

Fig. 1 Map of study sites.

Insert indicates general location of study sites relative to Ontario and Minnesota. *Triangles* represent NOAA or Environment Canada weather stations



Capture and collaring procedures followed Canadian Council on Animal Care Guidelines and were approved by the Ontario Ministry of Natural Resources and Forestry Animal Care Committee. Collars recorded animal location at approximately 4-h intervals throughout the year, with frequent gaps between 4-h fixes. We resampled these data to achieve a consistent 4-h fix rate ± 0.25 h. For more details, see Rodgers et al. (1996) and Street et al. (2015). Captures in Minnesota took place from January 22 to February 9, and in Ontario from January 24 to February 22, in each year in the respective datasets.

We included only 3-dimensional, differentially corrected fixes with horizontal dilution of precision (HDOP) <10 , providing a location accuracy of 3–7 m

(Rempel and Rodgers 1997). We removed all fixes within 24 h of collar deployment or following animal mortality as reported by the collar and verified by field technicians. We limited our analysis to summer (June 1–September 30) to achieve in-season constancy in habitat covariates such as flowering phenology (Street et al. 2015). After resampling, 8077 fixes (range per individual = 1–442, mean = 79) remained for 98 individuals in Ontario, and 112,057 fixes (range per individual = 13–1983, mean = 837) remained for 134 individuals in Minnesota. We excluded animals with fewer than 10 fixes (34 animals), giving a total of 120,134 fixes from 198 adult female moose for this analysis.

We created an aggregate land cover classification system using the Ontario Provincial Land Cover 2000

(OLC; Anonymous 2004), available from Land Information Ontario (LIO; <https://www.javacoeapp.lrc.gov.on.ca/geonetwork/srv/en/main.home>), and the U.S. National Land Cover Database 2011 (NLCD; Jin et al. 2013), available from the Multi-Resolution Land Characteristics Consortium (MRLC; <http://www.mrlc.gov/nlcd2011.php>), at 25 and 30 m² resolutions, respectively. We aggregated OLC and NLCD land cover classifications into common classifications based on similarities in class descriptions (Table A1 in supplementary materials). Land cover datasets may differ in their definitions of cover types; the primary difference between our OLC and NLCD datasets is how forest types are classified. For example, OLC named forest stands (i.e., deciduous, coniferous) are considered dense forest, and non-dense forests are lumped into a “sparse forest” category that may be either predominately deciduous or coniferous, but no distinction is made based on dominant vegetation type (Anonymous 2004). NLCD forest types are classified based on >20 % coverage by trees and a 75 % threshold in coverage by conifer or deciduous trees, and the NLCD has no analogous sparse cover classification (Jin et al. 2013), prohibiting the use of the OLC sparse category here. As such, the Ontario dataset may have a lower abundance of deciduous habitat than might be represented on the landscape based on the prevalence of sparse deciduous forests. To examine whether aggregated land cover classes were informative, we conducted cross validation of models of habitat selection (see “Analysis” section, below). Additionally, habitats can generally be ranked in terms of foraging quality based on the availability of deciduous foliage (Peek et al. 1976; Belovsky 1981), but the use of remotely sensed land cover data clearly prohibits an explicit evaluation of habitat quality.

Although GPS collars recorded temperature (°C), these data are biased by numerous factors such as position on the animal, pelage, activity level, heat loss via radiation, etc. (Van Beest et al. 2012). Consequently, we associated used and available locations with ambient temperature data as recorded by the NOAA or Environment Canada weather station closest to a given fix in space and time. Ambient temperatures used in this fashion are highly correlated with collar data and better represent the conditions experienced by an individual that may influence habitat selection (Street et al. 2015). Used locations were on average 17.76 km (Minnesota range

0.41–72.46; Ontario range 25.57–127.90) from the nearest weather station and 0.13 h (Minnesota range 0–2.86; Ontario range 0.01–0.98) from the closest recorded weather observation in time.

Analysis

We estimated models of habitat selection using step-selection functions (SSFs; Thurfjell et al. 2014). SSFs use a case–control design wherein the occurrence of a case (i.e., a used fix/step) is conditional to the availability of controls (i.e., available locations where a fix was not recorded) selected based on where a fix could have occurred given the observed distributions of step lengths and turn angles. Used and available locations associated with each step form strata, and the SSF is estimated using conditional logistic regression. The SSF methodology thus constrains the availability of environmental covariates in time and space to the movement characteristics of an individual and is commonly used to study animal movement in heterogeneous landscapes (Forester et al. 2009; Thurfjell et al. 2014).

Selecting available points using the empirical (i.e., observed) distributions of step lengths and turn angles requires three sequential fixes at the defined fix rate, and frequent gaps in a dataset will reduce the frequency of these instances. We selected available points using parametric distributions of step length between two consecutive fixes and bearing relative to true north (Forester et al. 2009). We estimated the rate of exponential decay (λ) of the observed step lengths of each individual and sampled step lengths from an exponential distribution with the estimated λ . We sampled bearings from a uniform distribution from 0 to 2π . Sampling in this fashion naturally oversamples around the used point due to increasing area of a circular sector with increasing distance from the used point (i.e., hyperdispersion of points increases with distance from the centroid). We transformed sampled step length l as the square root of the ratio between the observed sampled step length and the maximum sampled step length for an individual times the squared maximum sampled step length, $l = \sqrt{(l_{\text{observed}}/l_{\text{max}}) \times l_{\text{max}}^2}$, to correct for oversampling. We paired 10 available points to each used fix (i.e., 11 points per stratum). We modeled the probability of selecting each point within a stratum as a

function of proportional coverage of each of 5 land cover types (deciduous, coniferous, mixedwood, water, and treed wetlands) within a circular 50 m radius buffer. Other land cover types (e.g., grassland, developed) typically comprised 12 % of buffers but were not explicitly included in the regression (i.e., they serve as a reference category for the other cover types). We also included the step length (i.e., distance between consecutive fixes) to accommodate any bias introduced by using parametric distributions of step length (Forester et al. 2009). We included interactions between these main effects and (1) temperature ($^{\circ}\text{C}$), centered on previously reported summer thresholds for heat stress in moose (i.e., 14°C ; Renecker and Hudson 1986) such that positive values represented temperatures likely exceeding moose thermal neutral zones, and (2) the time of day a fix was recorded, transformed using four circular time harmonics, $\sin(2\pi t/24)$, $\sin(4\pi t/24)$, $\cos(2\pi t/24)$, and $\cos(4\pi t/24)$, to assess changes in habitat selection across the diurnal cycle (Forester et al. 2009). Four time harmonics were included to accommodate individual variation in activity patterns—that is, an individual may be more active during peak light/dark periods than crepuscular periods, or may exhibit more than one or two peak periods of activity. Because both temperature and time of day were constant within strata, they were considered only as interaction terms. In total, we estimated six main effects and five interactions per main effect (i.e., 36 coefficients). This model structure permits evaluation of whatever temperature effect on habitat selection remains after accommodating the time of day effect on selection patterns.

We estimated these models separately for each individual animal in the Minnesota and Ontario datasets. Averaging the individual regression coefficients within a given dataset produced the population-level coefficients and confidence intervals reported in Table A2 in supplementary materials. Such averaging accommodates within-animal variation in habitat selection and approximates a mixed effects modeling approach (Fieberg et al. 2010) but effectively reduces sample size for any estimated model. If sample sizes are small or model predictors are highly correlated, the variance of coefficients estimated by standard statistical models is often quite large. Modern telemetry data are typically not sample size limited, but correlated variables compounded by the dynamic nature of animal relocation typically produce low explanatory

power of fitted models and unreliable out-of-sample prediction (Beyer et al. 2010). We thus fitted our SSFs using conditional logistic regression with lasso (least absolute shrinkage and selection operator; Reid and Tibshirani 2014). The lasso maximizes the likelihood of the data subject to a constraint, determined by an additional tuning parameter (s), that limits the summed absolute value of model coefficients (Tibshirani 1996). When s is very large (i.e., approaching infinity), the lasso produces coefficients identical to ordinary regression; at values of s approaching 0, the lasso reduces the coefficients of uninformative predictors. This framework offers an appealing alternative to model averaging when the number of predictors is large relative to effective sample size (Hooten and Hobbs 2015). We selected our model coefficients as the estimates minimizing the cross validation statistic (Reid and Tibshirani 2014).

We binned used and available locations by the hour in which a fix was recorded and the ambient temperature as reported by the nearest weather station. We calculated the average proportional cover by land cover types in used and available locations across bins to visualize how used and available cover change across both diurnal and temperature gradients in both study sites and fit lowess regressions to both used and available bins to generate smoothed proportional coverage curves (Figs. 2, 3). We also calculated model-based estimates of selection strength (i.e., log relative risk) for each land cover class across time of day and temperature, holding either temperature constant at the upper thermoneutral limit of moose (i.e., $\Delta T = 0^{\circ}\text{C}$; Renecker and Hudson 1986) for time of day plots, or time of day constant at noon (1200 h) for temperature plots (Figs. 4, 5). We ranked the predicted selection strength at a given time or temperature to evaluate changes in relative selection strength across the diurnal cycle and temperature gradient, including selection for land cover types not included in the models (i.e., predicted selection = 0).

Lastly, we conducted a separate cross validation procedure for our step selection models based on a comparison of observed use of land cover covariates versus expected values predicted by the models. Specifically, we:

1. Fit step selection models to data from either Ontario or Minnesota. The data used to fit the model correspond to “training data”. The data

Minnesota

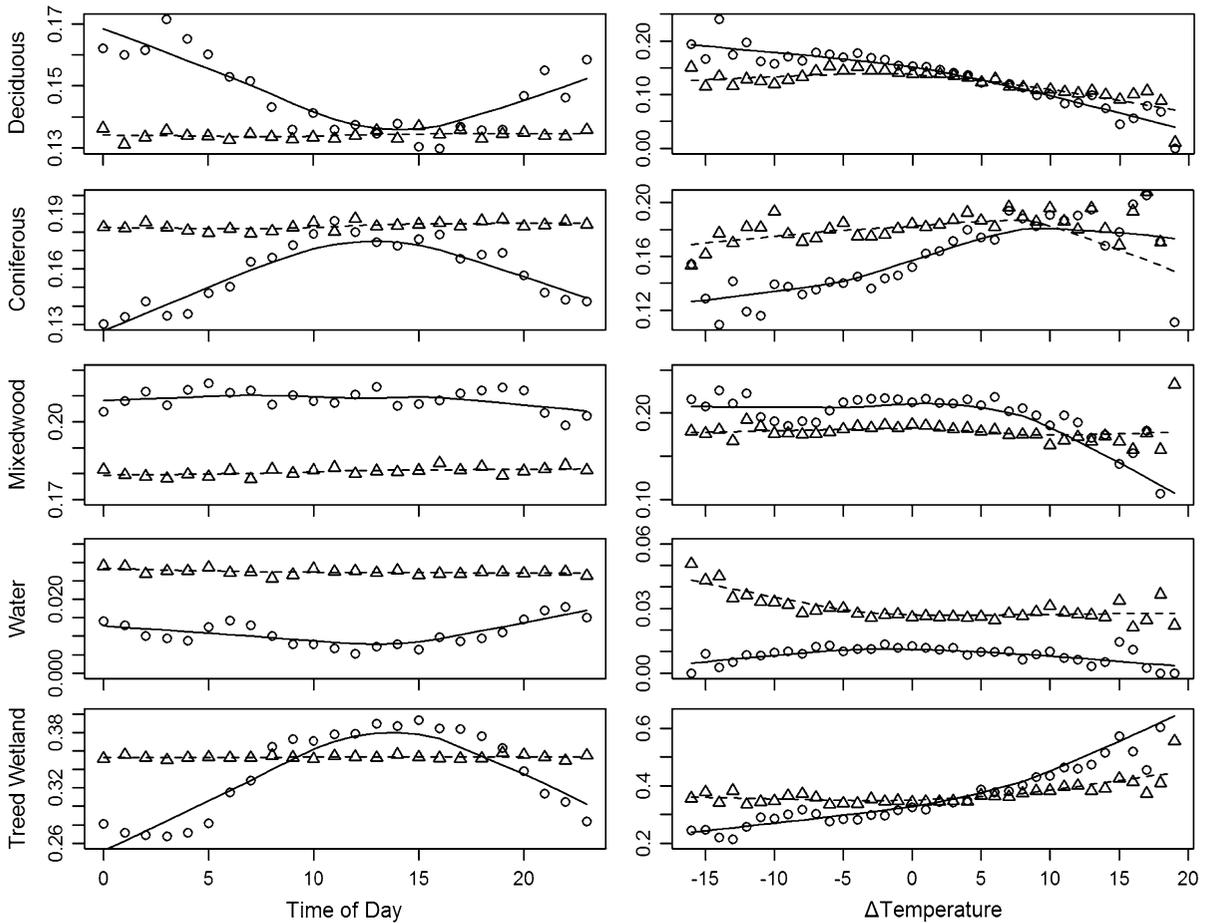


Fig. 2 Average used (solid lines and circles) and available (dashed lines and triangles) proportional representation within 50 m buffers of land cover classifications included in the analysis during summer (June 1–September 30) in Minnesota.

Patterns of use and availability change across both time of day (left column) and temperature (°C) scaled to moose (*A. alces*) upper thermal optima (right column)

that were not used in this step were treated as “test data”.

- Using the fitted model from step [1], we estimated the probability of choosing each location (both used and available) in the test data:

$$\hat{p}_{i,k} = \frac{\exp(x_{i,k}\beta)}{\sum_{j=1}^{11} \exp(x_{j,k}\beta)},$$

($i = 1, 2, \dots, 11, \quad k = 1, 2, \dots, n_{strata}$),

where i indexes a location (used or available) within stratum k , $x_{j,k}$ is a vector of covariate data for the j -th observation in stratum k , β is a vector of model coefficients, and n_{strata} gives the number

of strata (equivalent to the number of used locations).

Given the estimated probabilities $\hat{p}_{i,k}$, we calculated the expected proportional cover of the p -th land cover class in stratum k , $E[Z_k^p]$, as:

$$E[Z_K^p] = \sum_{i=1}^{11} \hat{p}_{i,k} Z_{i,k}^p,$$

where $Z_{i,k}^p$ is a scalar, representing the proportional cover of the p -th land-cover class associated with the i -th observation within stratum k .

We then plotted the average observed and expected values for each land-cover class across both

temperature and time of day to validate model fit across study sites (Figs. A1, A2, Supplemental Materials). Models and aggregated land cover were considered informative if observed patterns of use coincided with expectation.

All work was conducted using the base packages of Program R (R Core Team 2015) and the clogitL1 package (Reid and Tibshirani 2014).

Results

Cross validation of SSFs demonstrated high predictive accuracy of our models across sites (Figs. A1, A2 IN supplementary materials), indicating that the aggre-

gated land cover classification was informative and that our models capture variation in habitat selection across sites. We detected changes in proportional cover of land cover types associated with used locations in Minnesota across both time of day and temperature. On average, proportional cover of deciduous habitat decreased, and coniferous and treed wetland increased, in used locations at mid-day (Fig. 2). At all times of day deciduous cover was higher and coniferous cover lower in used locations compared to available locations. By contrast, treed wetland cover was greater at used relative to available locations only at mid-day, consistent with a switch toward positive selection for treed wetlands from approximately 0900–1800 h. Proportional coverage of

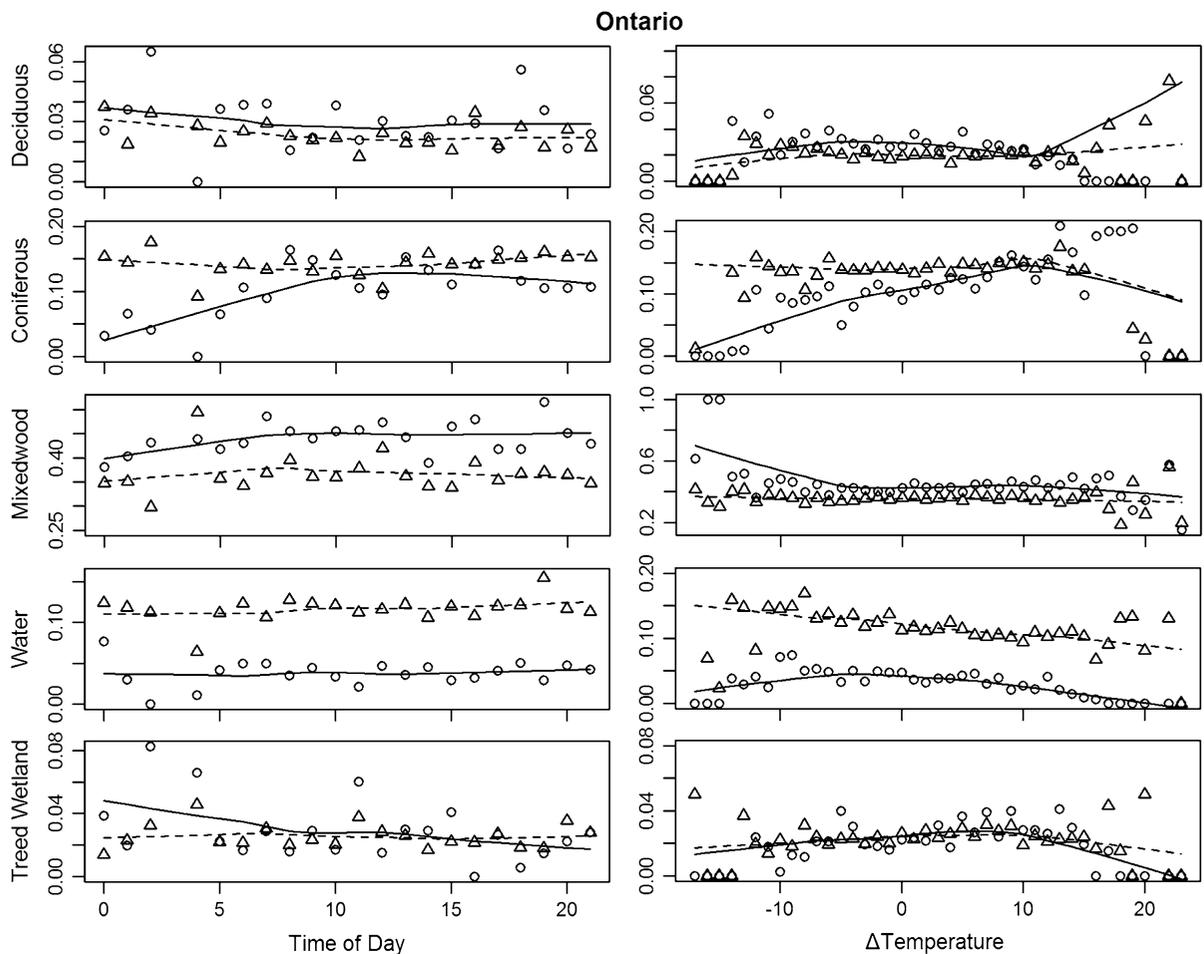


Fig. 3 Average used (solid lines and circles) and available (dashed lines and triangles) proportional representation within 50 m buffers of land cover classifications included in the analysis during summer (June 1–September 30) in Ontario.

Patterns of use and availability change across both time of day (left column) and temperature ($^{\circ}\text{C}$) scaled to moose (*A. alces*) upper thermal optima (right column)

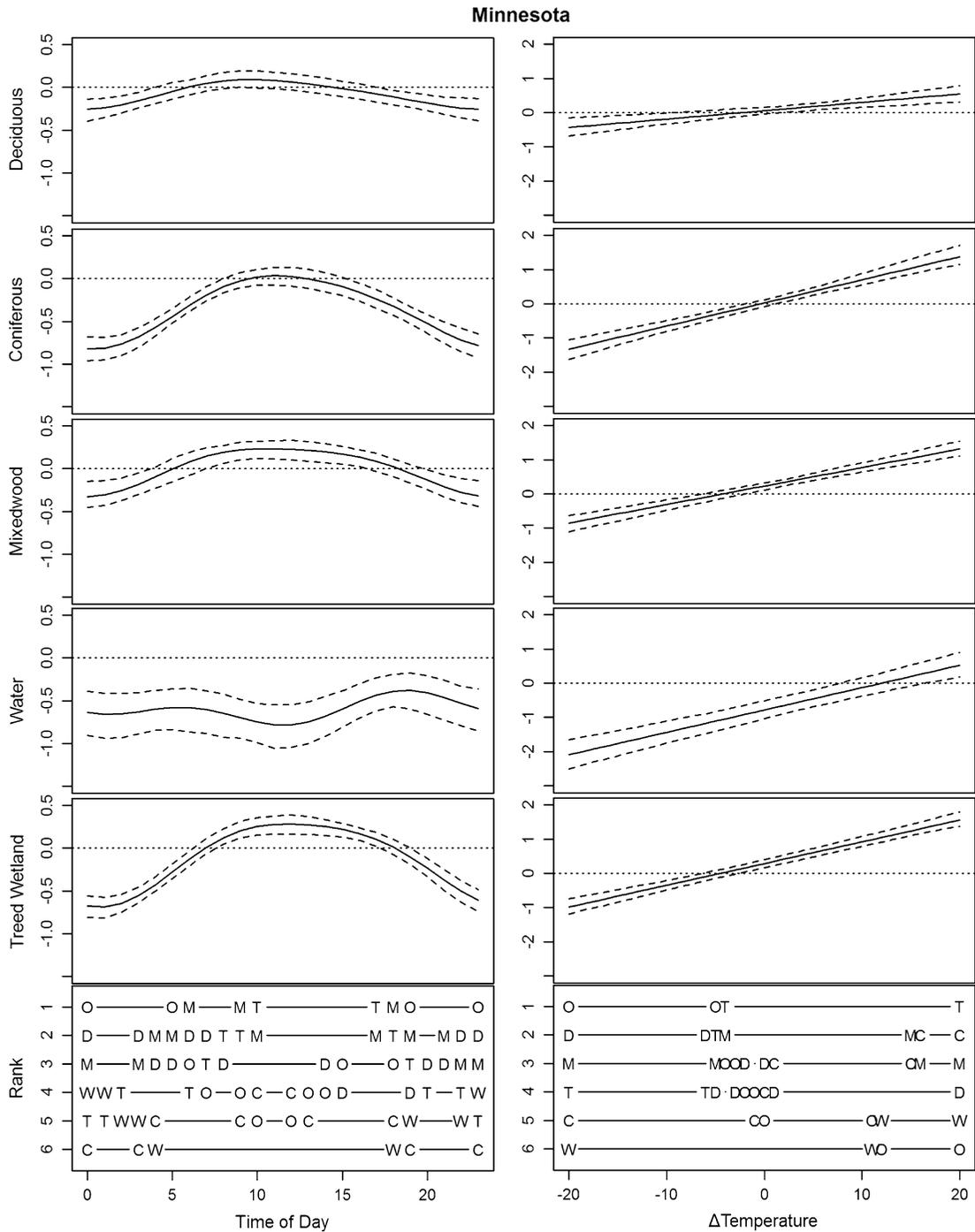


Fig. 4 Predicted selection strength (log relative risk, *solid lines*) by moose (*A. alces*) with 95 % Confidence Intervals (*dashed lines*) for 100 % cover by land cover classifications during summer (June 1–September 30) in Minnesota across both time of day (*left column*) and temperature (°C) scaled to moose upper thermal optima (*right column*). Temperature is held

constant at the moose upper thermal optimum (i.e., ΔTemperature = 0 °C) in time of day plots, and time is held constant at noon in ΔTemperature plots. *Bottom panels* indicate relative rank of selection strength for each land cover class (*D* deciduous; *C* coniferous; *M* mixedwood; *W* water; *T* treed wetland; *O* other) across the diurnal cycle and temperature gradient

deciduous and mixedwood associated with used locations declined with increasing temperature, and coniferous and treed wetlands increased with temperature. All four coverage types experienced a switch in directionality of use:availability ratios at temperatures exceeding approximately 10 °C above moose thermal optima (i.e., ~24 °C; Fig. 2).

Conversely, proportional cover associated with used and available locations in Ontario was less dynamic. We detected a marginal decline in treed wetland use and an increase in coniferous cover across time of day, but proportional cover at both used and available locations was otherwise consistent across the diurnal cycle (Fig. 3). Use of conifer increased, and use of mixedwood decreased, at warmer temperatures, but use of other land cover types exhibited little to no change in use across temperature. Thus between study sites we observed similar patterns of use of conifer with time of day, and conifer and mixedwood with temperature, but different patterns of use of deciduous and treed wetland habitats.

Predicted selection strength (i.e., log relative risk of selection) for the five land cover classifications was also inconsistent between sites. In Minnesota selection for deciduous, coniferous, mixedwood, and treed wetland stands notably increased at mid-day (Fig. 4). Despite increased selection strength for deciduous at mid-day, ranked relative selection strength was highest for mixedwood and treed wetland at mid-day (Fig. 4, bottom left), with ranked selection for deciduous declining at mid-day, consistent with our empirical findings (Fig. 2). Predicted selection strength for all cover types, relative to the “other” category (what was left out of the model, i.e., selection strength = 0), consistently increased across the temperature gradient (Fig. 4). Yet, ranked selection among all land cover types indicated declining selection for deciduous at high temperatures, and increased selection for treed wetlands, conifer, and mixedwood (Fig. 4, bottom right). Selection for water was consistently low across the temperature gradient.

In Ontario, predicted selection strength for water, deciduous, mixedwood, and treed wetland habitat was relatively invariant across time of day (Fig. 5). Selection for conifer increased at mid-day, consistent with predicted selection in Minnesota. Ranked selection strength indicated selection primarily favored mixedwood across the diurnal cycle, and selection for deciduous habitat peaked at night and early morning

(Fig. 5, bottom left). Ranked selection for conifer peaked at mid-day, but treed wetland, conifer, and water were generally avoided. Selection strength, relative to “other”, increased only for conifer and mixedwood habitat as temperature increased (Fig. 5). This pattern was also observed in ranked selection, with selection for conifer and mixedwood increasing across the temperature gradient (Fig. 5, bottom right).

Discussion

We found that habitat use by moose varied between two study sites differing primarily in latitude and landscape composition. In Ontario, proportional coverage of foraging stands (i.e., deciduous, mixedwood) was higher at used than available locations; non-foraging stand coverage was used less than its availability. In contrast, moose in Minnesota exhibited marked changes in patterns of habitat use across both time of day and temperature, most notably a decline in the use of deciduous and an increase in coniferous and treed wetland at mid-day. Use of these habitats may result in reduced foraging efficiency if quantity/quality of forage is lower than in deciduous stands (Peek et al. 1976; Belovsky 1981) and points to the importance of abiotic environmental conditions driving habitat selection patterns of moose in Minnesota. However, ranked selection was consistent between the two sites, with selection for conifer peaking around mid-day and at the highest temperatures. These results suggest that moose primarily select habitat during the summer based on foraging considerations (i.e., energy acquisition) except at the highest temperatures, consistent with previous studies (Peek et al. 1976; Belovsky 1981; Van Beest et al. 2012; Street et al. 2015).

We detected an increase in selection strength for all land cover types, relative to an “other” category (i.e., what was not in the model), as a function of temperature in Minnesota (Fig. 4). However, ranked selection declined for deciduous habitat and increased for coniferous, mixedwood, and treed wetlands with increasing temperature. This indicates a change in preference for habitat types across the temperature gradient and should produce a detectable shift in space use and distribution (Beyer et al. 2010), particularly at high temperatures. Indeed, at high temperatures moose in Minnesota used deciduous habitat

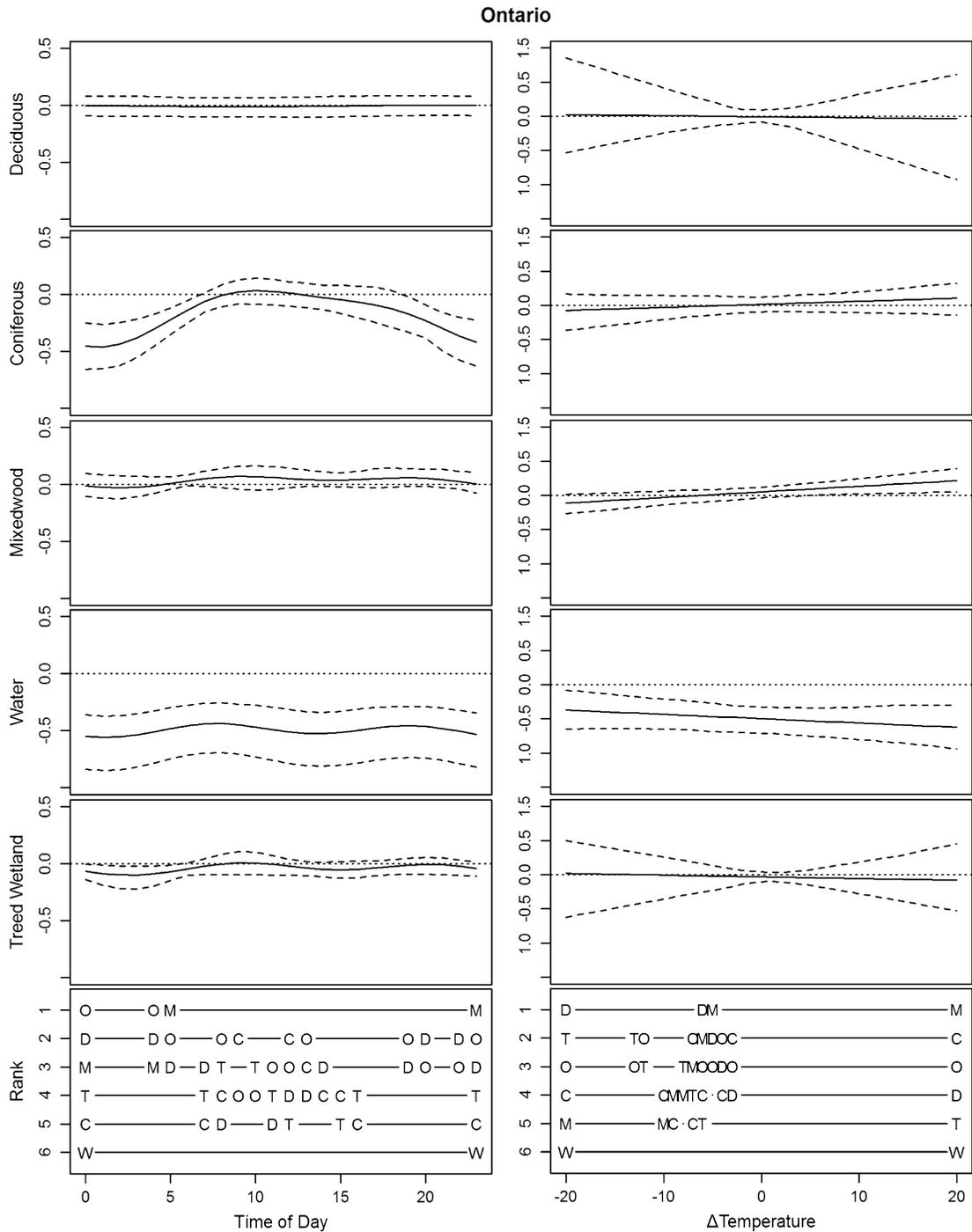


Fig. 5 Predicted selection strength (log relative risk, *solid lines*) by moose (*A. alces*) with 95 % confidence intervals (*dashed lines*) for 100 % cover by land cover classifications during summer (June 1–September 30) in Ontario across both time of day (*left column*) and temperature (°C) scaled to moose upper thermal optima (*right column*). Temperature is held

constant at the moose upper thermal optimum (i.e., ΔTemperature = 0 °C) in time of day plots, and time is held constant at noon in ΔTemperature plots. *Bottom panels* indicate relative rank of selection strength for each land cover class (*D* deciduous; *C* coniferous; *M* mixedwood; *W* water; *T* treed wetland; *O* other) across the diurnal cycle and temperature gradient

substantially less than at lower temperatures, favoring instead conifer and treed wetlands (Fig. 2). Thus at temperatures exceeding moose thermal optima (Reinecker and Hudson 1986), moose in Minnesota selected more strongly for those habitats providing thermal cover—that is, conifer and treed wetlands (Peek et al. 1976; Belovsky 1981; Van Beest et al. 2012; Street et al. 2015). It is worth noting that during the night and at low temperatures, the “other” category was actually most favored based on ranked selection (Figs. 4, 5), suggesting that during these periods moose may utilize habitat types not represented in our models (e.g., agricultural land, shrublands). These habitat types may provide additional foraging or bedding opportunities that we do not consider here, but universally these habitat types do not provide cover of any sort, which may be why they are favored at night when moose will be harder to detect and during cooler periods when temperatures are not limiting. Further research on selection and use of non-forested or “sub-prime” foraging habitats would further enlighten this observation, but we lack sufficient data to address this here.

If moose exposed to elevated ambient temperatures alter habitat selection toward thermal cover, why did moose in Ontario not substantially alter their habitat use (not selection) at high temperatures? Neither study site was substantially warmer on average than the other during data collection (15.8 and 14.6 °C in Ontario and Minnesota, respectively), but the two sites differ markedly in proportional coverage by land cover types. The Minnesota site contains a larger proportion of deciduous cover than Ontario, which in turn has twice as much dense mixedwood and very little deciduous cover (Figs. 2, 3). Reduced availability of deciduous habitat in Ontario may be attributable to differences in OLC and NLCD deciduous forest classification (see “Methods” section; Anonymous 2004, Jin et al. 2013), but the increased availability of dense mixedwood forest is telling. Mixedwood stands simultaneously provide foraging opportunities and thermal cover (Belovsky 1981; Van Beest et al. 2012), thus moose in high mixedwood landscapes such as Ontario are commonly in sufficient thermal cover regardless of time or location. These findings suggest that moose in Ontario are only forced to modify their habitat preferences under the most thermally stressful conditions (e.g., high temperatures at mid-day; Street et al. 2015), which is corroborated

by evidence that moose in southern Ontario typically do not exhibit the population declines expected at the southern extent of the species range (Murray et al. 2012). Conversely, the Minnesota site contains a greater abundance of deciduous vegetation, which provides ample vegetation but a less dense canopy, thus moose in this site have the opportunity to consistently select forage-rich environments that provide less thermal cover. Ironically, it is by virtue of living in a landscape of greater forage abundance that moose in Minnesota may experience foraging limitations under thermally stressful conditions, whereas moose in Ontario appear to be freed from this constraint.

The primary implication of these findings, then, is that foraging animals may experience a trade-off between acquiring resources (i.e., energy intake) and maintaining homeostatic conditions (i.e., energy expenditure) across environmental gradients (Speakman and Król 2010). Reducing net energy intake by reducing foraging opportunity has negative effects on animal fitness (Ritchie 1990; McLoughlin et al. 2006, 2007; Hodson et al. 2010), and selection for thermal cover in lieu of foraging habitat could result in reduced body mass and lifetime reproductive success (Van Beest et al. 2012; Monteith et al. 2015). However, recent research has demonstrated that reducing energetic expenditure by increasing efficiency of heat dissipation is an important component of endotherm fitness and may contribute more to net fitness in some circumstances than energy supply (Speakman and Król 2010). This seems particularly plausible if animals change their habitat preferences in response to landscape composition and abiotic conditions (i.e., habitat functional response; Myrsetud and Ims 1998). Under conditions of thermal stress, animals exhibiting a functional response for habitat selection might utilize thermal cover more frequently while simultaneously increasing selection strength for high quality foraging habitat to compensate. We detected a significant increase in selection for deciduous habitat both at high temperatures and at mid-day by moose in Minnesota (Fig. 4) concurrent with declining average use of deciduous habitat (Fig. 2), suggesting that the habitat functional response occurs as moose attempt to mitigate the potentially adverse effect of reduced foraging opportunity caused by increased use of thermal cover.

How successful this strategy may be is likely variable across landscapes. For example, Lenarz et al.

(2010) found that moose in Minnesota exhibit net negative population growth and suggested that this decline is attributable to increasing temperatures during winter. Monteith et al. (2015) found similar results for moose in the U.S. Rocky Mountains due to both increasing summer temperature and changes in flowering phenology. In contrast, moose populations in southern Ontario are on average stable or increasing (Murray et al. 2012), despite experiencing temperatures comparable to or greater than those described here or in other studies (Lenarz et al. 2010; Monteith et al. 2015). These studies state that changes in moose demographic rates and survivorship may be explained by changes in forage availability or increasing heat stress, but they agree that the precise mechanism driving population level responses to climate change requires investigation. We suggest that landscape configuration may be the mechanism explaining these discrepancies across regions. The functional response may permit reduction or negation of the fitness costs associated with reduced foraging habitat availability, allowing animals to modify behavior to both maximize energy intake and minimize energy expenditure given a certain environmental context. Although we focus on one example of how this functional response may arise—that is, through temperature-mediated changes in habitat selection—the habitat functional response could mitigate fitness loss due to any changes in space use. Our understanding of endotherm fitness would benefit from an explicit assessment of the contribution of discrete habitat types to fitness across changes in both landscape composition and abiotic conditions. Research synthesizing fitness and space use would permit investigation of explicit hypotheses of drivers of population decline across bioclimatic and latitudinal gradients and would be invaluable to the ecology and management of this and other species of concern.

Although the SSF methodology is now widely used in habitat selection and movement studies (Thurfjell et al. 2014), our use of the lasso is relatively uncommon in the ecological literature (Dahlgren 2010, but see Hooten and Hobbs 2015). The lasso places a cap on the sum of the absolute value of the regression coefficients and is a form of regression shrinkage (Tibshirani 1996; Dahlgren 2010; Reid and Tibshirani 2014). Shrinkage reduces the magnitude of regression coefficients associated with statistically unimportant variables and improves prediction

accuracy, a common shortcoming of habitat selection models (Fielding and Bell 1997; Beyer et al. 2010). Further, the lasso can be used for variable selection in cases where the number of parameters is large relative to effective sample size since uninformative predictors can be (and often are) reduced to 0 (Tibshirani 1996; Dahlgren 2010; Giudice et al. 2012). Currently, model selection in ecology is most often achieved using information theoretic criteria (Burnham and Anderson 2002; Stephens et al. 2005; Giudice et al. 2012). Although extremely flexible and useful when properly employed, some authors (e.g., Stephens et al. 2005, Giudice et al. 2012) have noted that over-reliance on information theoretic methods may lead to under-consideration of alternative hypotheses and ecological phenomena, instead depending on a suite of competing models to suggest ecological significance. Shrinkage estimators provide an alternative to information theoretic methods while requiring deeper consideration of the hypotheses and ecology under investigation. Given their utility and the availability of packages in most statistical software, these methods are a valuable tool for ecologists and managers and should be considered alongside other alternatives such as information theoretic methods (Babyak 2004; Giudice et al. 2012; Fieberg and Johnson 2015; Hooten and Hobbs 2015).

Investigating animal space use across spatially distinct regions allows researchers to investigate how environmental and geographic gradients influence habitat selection, space use, and movement patterns (Matthiopoulos et al. 2011). Here we demonstrate a strong effect of temperature, diurnal cycles, and landscape composition on patterns of habitat selection and space use, and identify a functional response in selection for foraging habitat driven largely by increased use of thermal cover. We suggest that the habitat functional response may serve as a mechanism mitigating fitness loss due to changes in space use which reinforces the importance of accommodating broad scale bioclimatic variation in studies of habitat selection. Habitat selection is context dependent, and similar models of habitat selection may produce markedly different realized patterns of space use driven by differences in landscape composition. Further studies examining how environmental factors (e.g., predator pressure, disturbance, weather) influence variation in fitness due to individual space use will provide insight into the ecological processes

driving population dynamics and distributions across spatiotemporal scales.

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