Can leaf vascular architecture help predict plant performance in a changing Arctic?

Mackenzie Lift

Abstract

A warming Arctic has been associated with shifts in vegetative cover. Plant functional traits have been used to explain why certain species do better than others, however, there is still great uncertainty. I plan to examine leaf vascular architecture to better understand why some plants species increase in cover with warming while others do not. Leaf venation traits will be collected from the common species in Utqiaġvik, Alaska from ambient and experimentally warmed plots along a soil moisture gradient and analyzed with vegetative cover data. Results are intended to show which venation traits are associated with plant success across different environmental conditions and will provide insight to the changing Arctic vegetation.

Introduction

Rising air temperatures as a result of of climate change are being seen on a global scale, but the most pronounced warming is seen at higher latitudes making the Arctic a useful predictor of future environmental change (AMAP, 2019; NRC, 2014). The Arctic is rapidly changing with diminishing sea ice (Serreze et al., 2003), thawing of permafrost (Osterkamp, 2007), and changes in the tundra vegetation (Pearson et al., 2013). Community change of tundra vegetation has been extensively studied (Callaghan et al., 2011; Chapin et al., 1995; Taylor et al., 2017) and its relationship with temperature change has been studied (Hollister et al., 2015; Walker et al., 2006) in long-term experiments through the International Tundra Experiment (ITEX). Shrub and graminoid cover is increasing while bryophyte and lichen cover is decreasing (Callaghan et al., 2011; Elmendorf et al., 2012), graminoids are growing taller (Hudson et al., 2011), and plants are greening earlier and longer (Hollister et al., 2015). Changes in the vegetative cover, plant growth, and plant phenology are due to a warming climate (Barrett et al., 2015; Hollister et al., 2015; Hudson et al., 2011; Walker et al., 2006), but what makes some plants more successful in different environments is uncertain.

Plant functional traits can possibly explain this uncertainty (Bjorkman et al., 2018; Myers-Smith et al., 2019). Plant functional traits are traits that can impact the fitness of the plant in terms of growth, reproduction, and survival (Violle et al., 2007). Leaf functional traits such as plant height, specific leaf area (SLA), leaf dry matter content (LDMC), leaf area, and leaf nitrogen content are related to the changing temperature and moisture levels of the Arctic (Bjorkman et al., 2018). Plant functional traits can alter both the functioning of the plant and the plant community in terms of photosynthetic rates, decomposition rates, canopy structure, freeze-thaw dynamics of soils, and trophic interactions (Myers-Smith et al., 2019). Therefore, leaf traits are influential to plant performance and community functioning and can provide insight into a changing Arctic. However, the connection between these leaf traits and the changing environment is not concrete and needs to be investigated further if plant success across different environments is to be understood.

To further investigate plant success, leaf venation traits need to be examined. Leaf vascular architecture is a “Rosetta Stone” for plant physiological functioning because it is a key determinant to photosynthetic rates, plant growth, and plant responses to environmental conditions (Sack & Scoffoni, 2013). Leaf venation traits include vein density, vein diameter, vein distance, and areole number and density. Venation traits provide a link between plant physiological functioning and climate (Blonder & Enquist, 2014). Among the traits, vein density is the most cogent and responsive to changes in soil moisture (Buckley et al., 2015; Roth-Nebelsick et al., 2001).

Vein density is measured as vein length per unit area (VLA) and enables higher hydraulic conductance. Plants in drier and warmer conditions will therefore perform better with greater vein densities (Sack & Scoffoni, 2013) as a result of VLA reducing the horizontal path length for water transportation to transpiring epidermis and increasing the amount of channels available for conduction (Buckley et al., 2015; Roth-Nebelsick et al., 2001). Plants in drier environments will also benefit from lower vein distance, which is a measure of spacing between the veins, because low distance implies greater productivity since there will be shorter pathways between veins and stomata (Blonder et al., 2011). Veins with greater diameters may have greater water and nutrient capacity and greater mechanical support (Mediavilla et al., 2020). The number of areolas, or regions of leaf tissue bounded by minor veins, can also be described by the number of vein loops in the venation architecture (Blonder et al., 2018; Buckley et al., 2015). The greater the number of areoles or closed loops, the more redundant pathways for water and nutrients to circumvent damage, improving leaf survivorship (Blonder et al., 2011; Sack & Scoffoni, 2013). Since areole density correlates with VLA, greater areole density can increase plant productivity, but can decrease leaf longevity (Sack & Scoffoni, 2013). Therefore, venation traits mentioned such as vein distance, vein diameter, and areole number and size can help explain variation in vein density between and within species.

Venation traits have been not been explored for Arctic vegetation and have not been extensively used to predict plant performance in a changing tundra community. Therefore, leaf vascular architecture needs to be explored in tundra plants because it can provide further insight as to why some plants are increasing more than others in different environments. The leaf vascular architecture of Arctic plants can be used to provide insight about the performance of plants in different community types. Then, I can see if the plants increasing in cover have certain leaf venation traits. The study will provide a better understanding of how leaf vascular architecture is associated with plant physiological performance.

Research Questions/Objectives/Hypotheses

I plan to investigate if tundra species with certain vascular architecture are associated with different environments and if certain architectural traits allow a species to capitalize on a warming environment. I will answer this question by 1.) determining the leaf vascular architecture of the common graminoids and several other species along a soil moisture gradient in a high Arctic tundra site (Utqiaġvik, Alaska), 2.) determining what species are increasing in cover in experimentally warmed plots, and 3.) examining the relationship of venation traits to warmer/colder and drier/wetter conditions to see what plants excel under what conditions. I hypothesize that leaf vascular architecture will vary between drier and wetter environments with increased vein density at drier sites. I also hypothesize that experimentally warmed plots will have a greater abundance of species with venation traits that favor warming, such as increased vein density. Lastly, I hypothesize that over years, years with increased warming and drier conditions will favor plants with increased vein density, while years with colder and wetter conditions will favor plants with lower vein densities. Since there is a lack of literature concerned with leaf venation traits of Arctic plants, this study will significantly contribute to literature on venation traits. Exploring venation traits in Arctic plants will enhance the scientific community's understanding of Arctic plants and how they will respond to a changing Arctic.

Methods

Study Area

Utqiaġvik, Alaska, (71°19’N, 156°24’W) (Fig 1) is a peninsula surrounded by the Arctic Ocean and has a mean summer temperature of approximately 4°C. The vegetation in Utqiaġvik was historically considered high Arctic tundra due to a lack of erect shrubs; while that is slowly changing, the area is still predominantly composed of graminoid species. In Utqiaġvik, a dry heath study site was established in 1994 and a wet meadow study site was established in 1995 (Fig 2). Each site consists of 24 control plots and 24 experimental plots following protocols established by the International Tundra Experiment (ITEX) (Molau & Mølgaard, 1996). Each plot is approximately 1m^2; experimental warming is achieved using open top chambers (OTCs) made from fiberglass which raises the temperature of the plot by 1-3°C (Fig 1; Hollister et al., 2006). The ITEX study sites are nested within the 1 km^2 Arctic System Science (ARCSS) Grid established in the early 1990’s. In 2010, 30 plots (separate from the ITEX plots) within the Grid, referred to as ARCSS Subset, are sampled annually to monitor changes in vegetation and the physical environment across the landscape (Fig 2). The dominant species of Utqiaġvik are *Carex aquatilis, Eriophorum spp., Poa arctica,* and *Salix rotundifolia*.

Figure 1. Top: Location of study sites in Utqiaġvik, Alaska. Bottom: Open top chamber on experimentally warmed plot.

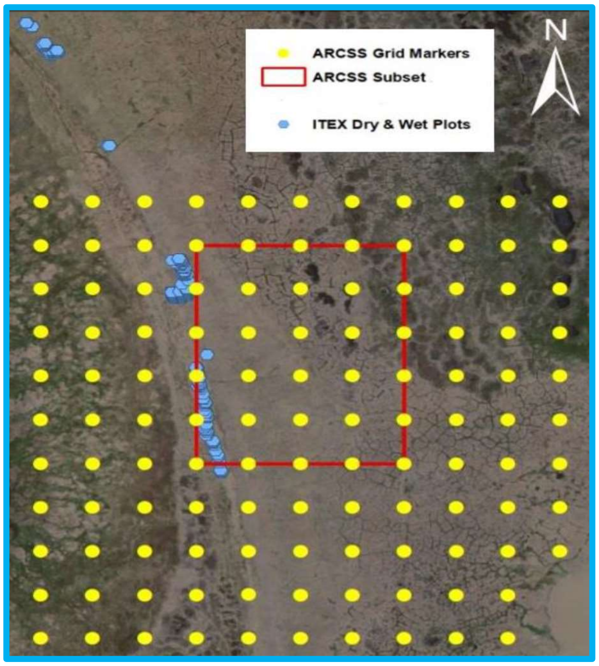

Figure 2. Arrangement of plots in Utqiaġvik, Alaska including the ARCSS Grid, ARCSS Subset and ITEX plots. Figure provided by Dr. Sergio Vargas.

Vegetative Cover Sampling

A cover assessment of the changing vegetation in Utqiaġvik, Alaska is sampled using the point intercept method. The non-destructive method outlined in the ITEX manual (Molau and Mølgaard, 1996; May & Hollister, 2012) uses a 75 cm x 75 cm grid with 100 intersection points (Fig 3). At each point, a ruler is lowered and starting at the canopy, every encounter is recorded along with the contact's height. Vascular plants are identified to species along with its alive/dead status. Sampling of an individual plot is conducted within the same two-week period across years to minimize differences in plant development across sampling years. ITEX site plots were sampled in years 1995 or 1996, 2000, 2008, 2012, and 2017; the ARCSS Subset plots were sampled annually in years from 2010 to 2019 (except for no sampling conducted in 2011).

Fig 3. Point frame grid used for non-destructive cover estimate of vegetation.

Venation Architecture Sampling

The species sampled will be *Carex aquatilis, Eriophorum angustifolium, Eriophorum russeolum, Arctophila fulva, Poa arctica, Dupontia fisheri, Alopecurus alpinus, Arctagrostis latifolia, Luzula confusa, Luzula arctica, Petasites frigidus, Salix pulchra* and *Salix rotundifolia.* For each species, five healthy basal leaves will be collected 1 leaf per plot at each site and each treatment. To analyze the vascular architecture and collect venation traits of each leaf, I will use the image analysis software phenoVein introduced by Bühler et al. (2015). To get an image of each leaf suitable for this software, we will clear each leaf prior to image acquisition by first immersing the leaves in a mixture of formaldehyde-acetic acid-alcohol (FAA) to fix the leaves. Then we will boil the leaves in 70% ethanol for one ten minutes followed by rinsing the leaves in distilled water and boiling the leaves in 85% lactic acid (Ueno et al., 2006). After the leaves cool, we will rinse them with distilled water twice and store them in Canada balsam. Each leaf will be examined under a dissecting scope. Using a dissecting scope digital camera, we will take an image of each leaf in a dark room with only transmitted background illumination via a condensed light source. We will then crop each image so that the background is blank space and load the image into phenoVein for leaf vein segmentation and analysis. We will use the image analysis software phenoVein introduced by Bühler et al. (2015) to analyze the vascular architecture of each leaf.

Statistical Analyses

We will perform a one-way Analysis of Variance (ANOVA) test to identify which venation traits were different across the plant species and soil moisture gradients. A nested ANOVA will be used to quantify which traits vary the most across the different species and soil moisture gradients. To analyze the cover data to see which species are changing over time, we will use a repeated measures ANOVA for each species. To see which traits are associated with cover change, we will use a Canonical Correspondence Analysis (CCA).

Plans for Dissemination

I will be presenting the above information at an International Tundra Experiment (ITEX) Conference tentatively scheduled for the fall of 2022; which was delayed due to the COVID-19 pandemic. My results will also be included in ongoing outreach activities of the research groups. In addition, I will also be presenting my research at the Student Scholars Day events hosted by Grand Valley State University. I am also planning to publish a manuscript in a reputable journal (to be determined) that outlines the major findings of my objectives.

Project Timeline

Semester	**Tasks to be Completed**
Winter 2021	Write research proposal and present to committee members Organize methods Perform preliminary analysis on preexisting data
Summer 2021	Collect leaf venation trait data for thesis at Utqiaġvik
Fall 2021	Analyze leaf venation trait data Begin writing thesis
Winter 2022	Finish writing thesis Defend thesis Graduate with Master’s in Biology

Budget

Item	**Justification**	**Provided by Project – Approximate Cost**	**Requested funding – Approximate Cost**
Airfare	Trip to Alaska is necessary to collect field data. Grand Rapids, MI to Utqiaġvik, AK	$1,600	
Per diem	Food in Utqiaġvik, AK is estimated at $39 per person per day for 90 user days	$3,510	
Vehicle	Needed for transportation in Utqiaġvik, AK	Provided by logistics provider	
Lab Space	Needed for carrying out methodology.	Provided by logistics provider	
Housing	Housing during stay in Utqiaġvik, AK	Provided by logistics provider	
Hot Plate	Needed for leaf clearing		$180
1L 70% Ethanol	Needed for leaf clearing		$40
1L 85% Lactic Acid	Needed for leaf clearing		$170
1L Formaldehyde-Acetic Acid-Alcohol (FAA)	Needed for leaf clearing		$24
100mL Canada Balsam	Needed for leaf storage		$120
Dissecting Scope with Digital Camera	Needed for image capture of leaves for image analysis		$750
Total		> $5,100	$1,284

Literature Cited

AMAP. (2019). *AMAP climate change update 2019: an update to key findings of snow, water, ice and permafrost in the Arctic (SWIPA) 2017*.

Barrett, R. T. S., Hollister, R. D., Oberbauer, S. F., & Tweedie, C. E. (2015). Arctic plant responses to changing abiotic factors in northern Alaska; Arctic plant responses to changing abiotic factors in northern Alaska. *American Journal of Botany*, *102*(12), 517–896. https://doi.org/10.3732/ajb.1400535

Bjorkman, A. D., Myers-Smith, I. H., Elmendorf, S. C., Normand, S., Rüger, N., Beck, P. S. A., Blach-Overgaard, A., Blok, D., Cornelissen, J. H. C., Forbes, B. C., Georges, D., Goetz, S. J., Guay, K. C., Henry, G. H. R., HilleRisLambers, J., Hollister, R. D., Karger, D. N., Kattge, J., Manning, P., … Weiher, E. (2018). Plant functional trait change across a warming tundra biome. *Nature*, *562*(7725), 57–62. https://doi.org/10.1038/s41586-018-0563-7

Blonder, B., & Enquist, B. J. (2014). Inferring climate from angiosperm leaf venation networks. *New Phytologist*, *204*(1), 116–126. https://doi.org/10.1111/nph.12780

Blonder, B., Salinas, N., Bentley, L. P., Shenkin, A., Chambi Porroa, P. O., Valdez Tejeira, Y., Boza Espinoza, T. E., Goldsmith, G. R., Enrico, L., Martin, R., Asner, G. P., Díaz, S., Enquist, B. J., & Malhi, Y. (2018). Structural and defensive roles of angiosperm leaf venation network reticulation across an Andes-Amazon elevation gradient. *Journal of Ecology*, *106*(4), 1683–1699. https://doi.org/10.1111/1365-2745.12945

Blonder, B., Violle, C., Bentley, L. P., & Enquist, B. J. (2011). Venation networks and the origin of the leaf economics spectrum. *Ecology Letters*, *14*(2), 91–100. https://doi.org/10.1111/j.1461-0248.2010.01554.x

Buckley, T. N., John, G. P., Scoffoni, C., & Sack, L. (2015). How does leaf anatomy influence water transport outside the xylem? *Plant Physiology*, *168*(4), 1616–1635. https://doi.org/10.1104/pp.15.00731

Bühler, J., Rishmawi, L., Pflugfelder, D., Huber, G., Scharr, H., Hülskamp, M., Koornneef, M., Schurr, U., & Jahnke, S. (2015). Phenovein—a tool for leaf vein segmentation and analysis. *Plant Physiology*, *169*(4), 2359–2370. https://doi.org/10.1104/pp.15.00974

Callaghan, T. V., Tweedie, C. E., Åkerman, J., Andrews, C., Bergstedt, J., Butler, M. G., Christensen, T. R., Cooley, D., Dahlberg, U., Danby, R. K., Daniëls, F. J. A., De Molenaar, J. G., Dick, J., Mortensen, C. E., Ebert-May, D., Emanuelsson, U., Eriksson, H., Hedenås, H., Henry, G. H. R., … Zemtsov, V. A. (2011). Multi-decadal changes in tundra environments and ecosystems: Synthesis of the international Polar year-back to the future project (IPY-BTF). *Ambio*, *40*(6), 705–716. https://doi.org/10.1007/s13280-011-0179-8

Chapin, F. S., Shaver, G. R., Giblin, A. E., Nadelhoffer, K. J., & Laundre, J. A. (1995). Responses of Arctic tundra to experimental and observed changes in climate. *Ecology*, *76*(3), 694–711. https://doi.org/10.2307/1939337

Elmendorf, S. C., Henry, G. H. R., Hollister, R. D., Björk, R. G., Bjorkman, A. D., Callaghan, T. V., Collier, L. S., Cooper, E. J., Cornelissen, J. H. C., Day, T. A., Fosaa, A. M., Gould, W. A., Grétarsdóttir, J., Harte, J., Hermanutz, L., Hik, D. S., Hofgaard, A., Jarrad, F., Jónsdóttir, I. S., … Wookey, P. A. (2012). Global assessment of experimental climate warming on tundra vegetation: Heterogeneity over space and time. In *Ecology Letters* (Vol. 15, Issue 2, pp. 164–175). Ecol Lett. https://doi.org/10.1111/j.1461-0248.2011.01716.x

Hollister, R. D., May, J. L., Kremers, K. S., Tweedie, C. E., Oberbauer, S. F., Liebig, J. A., Botting, T. F., Barrett, R. T., & Gregory, J. L. (2015). Warming experiments elucidate the drivers of observed directional changes in tundra vegetation. *Ecology and Evolution*, *5*(9), 1881–1895. https://doi.org/10.1002/ece3.1499

Hollister, R. D., Webber, P. J., Nelson, F. E., & Tweedie, C. E. (2006). Soil thaw and temperature response to air warming varies by plant community: Results from an open-top chamber experiment in northern Alaska. *Arctic, Antarctic, and Alpine Research*, *38*(2), 206–215. https://doi.org/10.1657/1523-0430(2006)38[206:STATRT]2.0.CO;2

Hudson, J. M. G., Henry, G. H. R., & Cornwell, W. K. (2011). Taller and larger: shifts in Arctic tundra leaf traits after 16 years of experimental warming. *Global Change Biology*, *17*(2), 1013–1021. https://doi.org/10.1111/j.1365-2486.2010.02294.x

May, J. L., & Hollister, R. D. (2012). Validation of a simplified point frame method to detect change in tundra vegetation. *Polar Biology*, *35*(12), 1815–1823. https://doi.org/10.1007/s00300-012-1224-1

Mediavilla, S., Martín, I., & Escudero, A. (2020). Vein and stomatal traits in leaves of three co-occurring Quercus species differing in leaf life span. *European Journal of Forest Research*, *0123456789*. https://doi.org/10.1007/s10342-020-01290-8

Molau, U., & Molgaard, P. (n.d.). *International Tundra Experiment (ITEX) Manual*.

Myers-Smith, I. H., Thomas, H. J. D., & Bjorkman, A. D. (2019). Plant traits inform predictions of tundra responses to global change. *New Phytologist*, *221*(4), 1742–1748. https://doi.org/10.1111/nph.15592

NRC. (2014). Arctic matters: The global connection to changes in the Arctic. In *Arctic Matters: The Global Connection to Changes in the Arctic*. National Academies Press. https://doi.org/10.17226/21717

Osterkamp, T. E. (2007). Causes of warming and thawing permafrost in Alaska. *Eos, Transactions American Geophysical Union*, *88*(48), 522–523. https://doi.org/10.1029/2007EO480002

Pearson, R. G., Phillips, S. J., Loranty, M. M., Beck, P. S. A., Damoulas, T., Knight, S. J., & Goetz, S. J. (2013). Shifts in Arctic vegetation and associated feedbacks under climate change. *Nature Climate Change*, *3*(7), 673–677. https://doi.org/10.1038/nclimate1858

Roth-Nebelsick, A., Uhl, D., Mosbrugger, V., & Kerp, H. (2001). Evolution and Function of Leaf Venation Architecture: A Review. *Annals of Botany*, *87*(5), 553–566. https://doi.org/10.1006/anbo.2001.1391

Sack, L., & Scoffoni, C. (2013). Leaf venation: structure , function , development , evolution , ecology and. *New Phytologist*, 983–1000.

Serreze, M. C., Maslanik, J. A., Scambos, T. A., Fetterer, F., Stroeve, J., Knowles, K., Fowler, C., Drobot, S., Barry, R. G., & Haran, T. M. (2003). A record minimum arctic sea ice extent and area in 2002. *Geophysical Research Letters*, *30*(3). https://doi.org/10.1029/2002GL016406

Taylor, P. C., Maslowski, W., Perlwitz, J., & Wuebbles, D. J. (2017). Arctic Changes and their Effects on Alaska and the Rest of the United States. *Fourth National Climate Assessment*, *I*. https://doi.org/10.7930/J00863GK

Ueno, O., Kawano, Y., Wakayama, M., & Takeda, T. (2006). Leaf Vascular Systems in C3 and C4 Grasses: A Two-dimensional Analysis. *Annals of Botany*, *97*(4), 611–621. https://doi.org/10.1093/aob/mcl010

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! In *Oikos* (Vol. 116, Issue 5, pp. 882–892). Blackwell Publishing Ltd. https://doi.org/10.1111/j.0030-1299.2007.15559.x

Walker, M. D., Wahren, C. H., Hollister, R. D., Henry, G. H. R., Ahlquist, L. E., Alatalo, J. M., Bret-Harte, M. S., Calef, M. P., Callaghan, T. V., Carroll, A. B., Epstein, H. E., Jónsdóttir, I. S., Klein, J. A., Magnússon, B., Molau, U., Oberbauer, S. F., Rewa, S. P., Robinson, C. H., Shaver, G. R., … Wookey, P. A. (2006). Plant community responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(5), 1342–1346. https://doi.org/10.1073/pnas.0503198103