ROOT PHENOLOGY IN A CHANGING WORLD

A Dissertation in
Ecology

by
Laura Radville
© 2017 Laura Radville

Submitted in Partial Fulfillment
of the Requirements
for the Degree of
Doctor of Philosophy

May 2017
The dissertation of Laura Radville was reviewed and approved* by the following:

David M. Eissenstat  
Professor of Woody Plant Physiology  
Graduate Program Chair, Intercollege Graduate Degree Program in Ecology  
Dissertation Advisor  
Chair of Committee

Eric Post  
Professor of Wildlife, Fish, and Conservation Biology

Margot Kaye  
Associate Professor of Forest Ecology

Kathleen M. Brown  
Professor of Plant Stress Biology

*Signatures are on file in the Graduate School
Shifts in plant phenology have been widely reported in response to global warming, and they can have strong effects on ecosystem processes and greenhouse gas emissions. Temperature is widely cited as the most important factor controlling the timing of aboveground growth, but the effects of warming on belowground phenology are poorly understood. Because root and shoot phenology may not be synchronous and may not be influenced by warming in the same way, root phenology research is critical to understanding shifts in whole-plant growth with climate change.

In order to more fully understand the drivers of root phenology, in Chapter 1 we conducted a literature review of root phenology studies and determined that only 29% of these studies examined phenology in a highly quantitative way. We propose that if future studies quantitatively examine different phases of annual root phenology separately, specifically root initiation, peak, and cessation, we may be able to better understand the drivers of root phenology. In Chapter 2, we carried out this idea by quantitatively examining root initiation, peak, and cessation in a long-term dataset of grape root phenology. We determined that drivers of phenology above- and belowground are not likely to be the same, and drivers may change throughout the year. In Chapter 3 we examined the influence of experimental warming and herbivore exclusion on phenology in the Arctic. In this study, we found that warming did not affect roots in the same way as leaves, because warming advanced leaf phenology but not root phenology. We conducted a similar experimental warming study in Chapters 4-5, in which we compared the influence of warming on two vegetation types. Warming had minimal impacts on phenology above- and belowground at this site. Additionally, in contrast to expectations, root growth was not a primary driver of carbon exchange despite high root:shoot ratios at this site. Because direct drivers of root phenology are still unclear, future efforts should continue this line of research and use manipulative, controlled studies.
TABLE OF CONTENTS

LIST OF FIGURES .......................................................................................................................... vi
LIST OF TABLES ............................................................................................................................... xi
ACKNOWLEDGEMENTS ................................................................................................................. xiii

Chapter 1  Root Phenology in a Changing Climate ................................................................. 1

Abstract ....................................................................................................................................... 1
Introduction ................................................................................................................................. 2
Background .................................................................................................................................. 4
Initiation of root growth ............................................................................................................ 6
Drivers of peak root growth ...................................................................................................... 7
Cessation of root growth: implications for a lack of winter dormancy ................................ 8
Potential impacts of climate change ......................................................................................... 9
The importance of depth ............................................................................................................. 11
Future directions and conclusions ......................................................................................... 12
References ................................................................................................................................. 28

Chapter 2  Limited linkages of above- with belowground phenology: A study in grape ........ 40

Abstract ....................................................................................................................................... 40
Introduction ................................................................................................................................. 41
Methods ..................................................................................................................................... 44
Results ....................................................................................................................................... 50
Discussion ................................................................................................................................... 53
References ................................................................................................................................... 69

Chapter 3  Root phenology in an Arctic shrub-graminoid community: The effects of long-
term warming and herbivore exclusion ............................................................................. 75

Abstract ....................................................................................................................................... 75
Introduction ................................................................................................................................. 77
Methods ..................................................................................................................................... 79
Results ....................................................................................................................................... 84
Discussion ................................................................................................................................... 86
Conclusions ................................................................................................................................. 90
References ................................................................................................................................... 97

Chapter 4  On the sensitivity of root and leaf phenology to warming in the Arctic .............. 103

Abstract ....................................................................................................................................... 103
Introduction ................................................................................................................................. 104
Methods ..................................................................................................................................... 105
Results ....................................................................................................................................... 109
Discussion ................................................................................................................................... 111
LIST OF FIGURES

Figure 1-1: Potential controls over root phenology. Solid lines indicate direct controls and dashed lines indicate indirect controls on root phenology. Gray boxes represent endogenous controls; white boxes represent exogenous controls. PPFD is photosynthetically active flux density. ........................................................................... 13

Figure 1-2: Seasonal shoot and root growth in two deciduous species and two evergreen species in the Eberswalde root laboratory in Germany. Shoot growth is indicated by the dotted area and root growth by the solid area. Figure is adapted from Lyr and Hoffman (1967). ........................................................................................................ 14

Figure 1-3: Root elongation at different photoperiods. A. Average root elongation (mm day$^{-1}$) at decreasing day lengths from July to September in mixed vegetation plots in Kangerlussuaq, Greenland. Error bars indicate standard error of the mean (n=24 at all day lengths). B. Average root elongation (mm day$^{-1}$) of Dupontia fischeri at decreasing day lengths in Barrow, Alaska, U.S.A. Photoperiod was experimentally controlled in phytotrons. Error bars indicate standard error of the mean (n=120 at 24h, n=24 at 21h, and n=6 at 18h). Figure adapted from Shaver and Billings (1977). ............................................................................. 14

Figure 1-4: Seasonal root production at three 10 cm depth intervals. A. Average daily root production (mm day$^{-1}$ standardized to a 50 cm$^2$ observation window) in mixed vegetation plots in the Arctic near Kangerlussuaq, Greenland. The timing of root production was not significantly different between soil depths, but soil temperature was significantly different between soil depths. B. Average daily root production (mm day$^{-1}$ in 50 cm$^2$ observation window) in Artemisia tridentata in Curlew Valley, Utah, U.S.A. Adapted from Fernandez and Caldwell (1975). ............................................................................ 15

Figure 2-1: Seasonal root standing crop patterns in ‘Concord’ grape, Vitis labruscana Bailey, in Fredonia, New York, USA, for two pruning treatments (heavy and minimal with irrigation treatments combined) over five years. Proportion of seasonal root standing crop is the length of roots present on a given date divided by the total root standing crop across the entire season. Standard errors were calculated from the length of roots present on a given date for a given block. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total length of roots (m m$^{-2}$) present between April and November........................................................................ 59

Figure 2-2: Seasonal root production in ‘Concord’ grape, Vitis labruscana Bailey, in Fredonia, New York, USA, for two pruning treatments (heavy and minimal with irrigation treatments combined) over five years. Proportion of seasonal root production is the length of new root extension on a given date divided by the total root extension in the entire season. Standard errors were calculated from the length of roots born on a given date for a given block. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total lengths of root extension (m m$^{-2}$) between April and November........................................................................ 60
Figure 2-3: Seasonal root standing crop patterns in ‘Concord’ grape, *Vitis labruscana* Bailey, in Fredonia, New York, USA, for two irrigation treatments (irrigated and not irrigated with pruning treatments combined) over five years. Proportion of seasonal root standing crop is the length of roots present on a given date divided by the total root standing crop over the season. Standard errors were calculated from the length of roots present on a given date for a given block. X-axis tick marks represent the first of the month. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total standing crop (length) of roots per unit observation surface of the minirhizotron (m m²) between April and November.

Figure 2-4: Seasonal new root production in ‘Concord’ grape, *Vitis labruscana* Bailey, in Fredonia, New York, USA, for two irrigation treatments (irrigated and not irrigated with pruning treatments combined) over five years. Proportion of seasonal root production is the length of root extension on a given date divided by the total length of root extension in the season. Standard errors were calculated from the length of roots born on a given date for a given block. X-axis tick marks represent the first of the month. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total lengths of root extension (m m²) between April and November.

Figure 2-5: The percent of new root growth that occurred during each of four periods related to aboveground phenophases over five years 1998 to 2002. Data come from ‘Concord’ grape, *Vitis labruscana* Bailey, in Fredonia, New York, USA with irrigation and pruning treatments combined. Error bars represent the minimum and maximum values over the five years, boxes represent the upper bound of the first and third quartiles, and middle line represents the median.

Figure 2-6: Seasonal root production patterns in ‘Merlot’ grape, *Vitis vinifera*, on two rootstocks 1103P and 101-14 Mgt. in Oakville, California, USA, over three years 2003-2005 under no irrigation (0% ETc) calculated as the length of root extension in a given block on a given date divided by the total length of root extension in each year. Standard errors were calculated from the mean percentages of roots born. X-axis tick marks represent the first of the month. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total length of root extension (m m²) in each year.

Figure 2-7: The percent of new root growth that occurred during each of four periods related to aboveground phenophases over three years from 2003-2005. Data come from ‘Merlot’ grape, *Vitis vinifera*, in Oakville, California, USA. Error bars represent maximum and minimum values and squares represent the median.

Figure 2-8: Relationships of root initiation (the date at which root growth first occurred in a given experimental unit), peak root standing crop (date with maximum length of roots), peak root growth rate (maximum length of new roots produced over a two week period), and root cessation (date at which there were at least three following sampling dates with less than 5% of total root growth) with days before or after bloom, day of year, and soil temperature. Points represent the average abiotic conditions that occurred on these key phenological states. Error bars represent 95%
confidence intervals. Data are from 1998-2002 in ‘Concord’ grape, Vitis labruscana Bailey, in Fredonia, New York, USA.

Figure 3-1: The proportion of maximum seasonal root (standing crop) and shoot (NDVI) growth for mixed-vegetation plots in 2013 and 2014. Black lines with circular points indicate the mean proportion of maximum root growth, and gray lines with square points represent the mean proportion of maximum shoot growth. All treatments are combined (N=24). Error bars indicate ±1 SE. The minimum and maximum root standing crops were 0.1 and 5.4 cm cm\(^{-2}\) in 2013 and 0.09 and 4.4 cm cm\(^{-2}\) in 2014. The minimum and maximum NDVIs were 0.07 and 0.88 in 2013 and 0.16 and 0.84 in 2014.

Figure 3-2: The proportion of maximum seasonal root (standing crop) and shoot (NDVI; leaf cover) growth for mixed-vegetation plots in 2013 and 2014, separated by warming and exclosure treatments. A. Proportion maximum shoot and root growth in 2013 and 2014, separated by exclosure treatment with warming treatments combined. Black dashed lines with closed circles indicate root standing crop with no herbivory, gray dashed lines with open circles are root standing crop with herbivory, gray solid lines with open squares are NDVI with herbivory, and black solid lines with closed squares are NDVI without herbivory. The timing of leaf cover was significantly different between plots with and without herbivory, but the timing of root standing crop was not. B. Proportion maximum shoot and root growth in 2013 and 2014, separated by warming treatment with exclosure treatments combined. Black dashed lines with closed circles indicate ambient root standing crop, gray dashed lines with open circles are warmed root standing crop, gray solid lines with open squares are warmed NDVI, and black solid lines with closed squares are ambient NDVI. The timing of leaf cover was significantly different between warmed and unwarmed plots, but the timing of root standing crop was not. Sample size (N) was 12 for each of the two treatments (Error bars indicate ±1 SE).

Figure 3-3: Grey area on left y-axis represents mean daily new root production (cm cm\(^{-2}\) day\(^{-1}\)) in mixed-vegetation plots in 2014, separated by soil depth. The date of peak root production was June 2 in 2014 and the average total root production from May 17 to September 5 2014 was 2.1±0.2 cm cm\(^{-2}\) (±SE). The black line on the right y-axis is the estimated soil temperature for each depth. Error bars indicate ±1 SE. All treatments are combined (N=24). Top row represent roots 1 to 10 cm below soil organic layer, second row represents roots 11-20 cm below soil organic layer, and bottom row represents roots 21-30 cm below soil organic layer. Figure is adapted from Radville et al. (2016).

Figure 3-4: A. The average daily volumetric soil water content from 0-10 cm in mixed-vegetation plots in 2013 and 2014. B. The average daily soil temperature at 5 cm in mixed-vegetation plots in 2013 and 2014. Error bars indicate ±1 SE and N=24.

Figure 4-1: A. The difference between daily maximum air temperature in warmed plot and daily maximum temperature in ambient plots (C). B. The difference between daily minimum air temperature in warmed plot and daily minimum temperature in ambient plots (C). In both panels, values are averages across all vegetation types. On
average across all years and vegetation types, open-top chambers warmed maximum daily temperatures by $2.2 \pm 0.1 \degree C$ (mean±SE) but had little effect on minimum daily temperature. Error bars represent standard error of the mean.

**Figure 4-2:** Relative leaf cover (NDVI on a given date/maximum NDVI in that year) averaged for ambient and warmed plots in 2015 and 2016. Dashed gray line and gray points are the mean of ambient plots and solid black line and points are the mean of warmed plots. There was no significant effect of the warming treatment. Error bars represent standard error of the mean.

**Figure 4-3:** Timing of relative new root production (root production on a given date/maximum root production in that year) in warmed and ambient plots for three growing seasons. The warming treatment did not have a significant effect on the timing of root production. Gray line and points are the mean of ambient plots and black line and points are the mean of warmed plots. Error bars represent standard error of the mean.

**Figure 4-4:** Timing of relative root standing crop (roots present on a given date/maximum root standing crop in that year) in warmed and ambient plots for three growing seasons. Gray line and points are the mean of ambient plots and black line and points are the mean of warmed plots. Error bars represent standard error of the mean.

**Figure 5-1:** The proportion of maximum root standing crop and leaf cover in 2014 and 2015, measured as NDVI, in graminoids (A), mixed graminoids and shrubs (B), and shrubs (C; *Betula nana*). Dotted gray line represents proportion of maximum root standing crop (root standing crop observed/maximum root standing crop in that year). Solid black line represents proportion of maximum NDVI (NDVI on that date/maximum NDVI in that year). Error bars indicate ±1 standard error of the mean ($N=16$).

**Figure 5-2:** Relative new root production (new roots produced · day$^{-1}$ on that date/maximum new roots produced · day$^{-1}$ in that season) from 2014 and 2015 in graminoids, mixed graminoids and shrubs, and shrubs (*Betula nana*). Solid gray lines represent graminoid plots, solid black lines represent shrub plots, and dotted black lines represent mixed plots. Error bars indicate ±1 standard error of the mean ($N=16$).

**Figure 5-3:** Carbon exchange in 2014 and 2015 in graminoid and shrub (*Betula nana*) plots, averaged for warmed and ambient treatments. Solid lines with filled black circles are shrub plots, and dashed lines with unfilled squares are graminoid plots. Gross ecosystem production (GEP) represents the net CO$_2$ produced (GEP = NEE – ER; negative values indicate carbon uptake), net ecosystem exchange (NEE) is net CO$_2$ exchange of the ecosystem at mid-day, and ecosystem respiration is the net CO$_2$ flux to the atmosphere (ecosystem carbon exchange in darkness). Negative values on the y-axis below the solid line indicate net CO$_2$ uptake by ecosystem, and positive values above the solid line indicate net CO$_2$ release to the atmosphere. Error bars represent ±1 standard error of the mean ($N=8$).
Figure 5-4: A. Lack of significant correlation between ecosystem respiration (ER) at 20°C and live root production. B. Lack of significant correlation between ER at 20°C and live root standing crop. C. Significant correlation between GEP at 20°C and NDVI. D. Significant correlation between ER at 20°C and NDVI. Dashed lines are derived from mixed models. Below the solid line there is a net CO₂ uptake by ecosystem, and above the solid line there is a net CO₂ release into the atmosphere.

Figure 5-5: A. The relationship between error in an ecosystem respiration (ER) model (observed ER - estimated ER) and root standing crop. The relationship is not significant. B. The relationship between ER model error and root production rate. The relationship is not significant. C. The significant relationship between ER model error and soil temperature at 10 cm. The dashed line represents the results of a mixed model of error and soil temperature, with plots repeated through time.

Figure 5-6: A. Root turnover from 2014-2015, separated by vegetation type (graminoids, mixed plots, and Betula nana shrub plots). Root turnover is estimated for first through third order, absorptive, roots. B. Live root biomass (g m⁻²) of first through third order roots estimated from harvest measurements in 2015. C. Aboveground biomass (g m⁻²) estimated from relationship between measured NDVI in 2015 and published relationship between NDVI and shoot biomass. Black bars represent graminoid plots, dotted bars represent mixed plots, and gray bars represent shrub (Betula nana) plots. Error bars represent ±1 standard error of the mean (N=8).

Figure 5-7: Root length density of Betula nana, graminoids, and mixed plots in 2015, separated by depth. Gray bars are the mean of graminoid plots, striped bars are the mean of mixed plots, and black bars are the mean of Betula plots. Error bars represent the standard error of the mean (N=8).

Figure 5-8: Comparison of our estimates of live root:shoot ratios to those from review of other tundra studies by Iversen et al. (2015). Root:shoot ratios in this study are generated from total live root biomass (all root orders) and aboveground biomass estimated from measured NDVI. Center line represents median, upper limit of box is 75th percentile, lower limit of box is 25th percentile, and error bars represent 10th and 90th percentiles (N=8 in this study).
**LIST OF TABLES**

Table 1-1: The number of studies that quantify the timing of events and found evidence of winter root growth. Only studies that used minirhizotron or rhizotron methods were examined for winter root growth measurements and quantification of root phenology. ........................................................................................................................................ 16

Table 1-2: List of literature reviewed with citation, location, climate, vegetation type, species, whether study used minirhizotron or rhizotron methods, whether study was quantitative or qualitative, and whether study found evidence of winter root growth. ... 17

Table 2-1: Percent of yearly new root production during periods related to aboveground phenology of *Vitis labruscana* in Fredonia, New York, USA. Data are from 1998-2002 and represent the mean ± standard error (SE), standard deviation (SD), and coefficient of variation (CV). Variation among years in percent root growth during each phenophase, as indicated by SD, was significant ($P < 0.05$). ............................................................................. 67

Table 2-2: Percent of yearly new root production during periods related to aboveground phenology of *Vitis vinifera* in Oakville, California, USA. Data are from 2003-2005 and represent the mean ± standard error (SE), standard deviation (SD), and coefficient of variation (CV). Variation among years in percent root growth during each phenophase, as indicated by SD, was significant ($P < 0.05$). ............................................................................. 67

Table 2-3: Interannual variation in shoot and root phenology across six studies. The variation in growing degree days (GDD) for dates of phenological events are also presented. On average, the timing of root phenology was more variable than aboveground phenology, and the variation in GDD at peak root phenology was greater than that for aboveground phenology. Variation in phenology is measured as variation in the date at which the phenological event occurred. C.V. represents coefficient of variation, and ‘N/A’ indicates that data were not available. ................. 68

Table 3-1: Correlations (Spearman’s ρ) with daily root production (cm cm$^{-2}$ day$^{-1}$). End of season represents July 29 to August 12 in 2013 and August 5 to September 5 in 2014.......................................................................................................................................... 95

Table 3-2: Peak date of root production is the date on which new root growth over the previous week was highest. Peak date of root standing crop is date with largest total length of roots. Peak date of vegetation cover is date with largest NDVI. The percent of maximum root production is a metric of seasonality, calculated as the new root growth on each date divided by the maximum root production in that plot and year. Percent of maximum standing crop and NDVI are calculated in the same way. “Warming” denotes warming/ambient treatments, “Exclosure” denotes herbivores present/absent, and “Depth” is a comparison of 0-10cm, 10-20cm, and 20-30cm......... 96
Table 5-1: Seasonal correlations (Spearman’s $\rho$) between plant phenology and carbon fluxes in 2014 and 2015. Leaf phenology is measured as NDVI on each date, and root phenology is root standing crop on each date. Gross ecosystem production (GEP) is CO$_2$ production ($\text{GEP} = \text{NEE} - \text{ER}$), net ecosystem exchange (NEE) is net CO$_2$ exchange at mid-day, and ecosystem respiration is net CO$_2$ flux to the atmosphere. *P<0.05; **P<0.01; ***P<0.001; ns, not significant. .......................... 149

Table 5-2: Seasonal correlations (Pearson) between ecosystem respiration and other environmental variables in 2014 and 2015. NDVI is normalized difference vegetation index, Gross ecosystem production (GEP) is CO$_2$ production ($\text{GEP} = \text{NEE} - \text{ER}$), net ecosystem exchange (NEE) is net CO$_2$ exchange at mid-day, and ecosystem respiration is net CO$_2$ flux to the atmosphere. *P<0.05; **P<0.01; ***P<0.001; ns, not significant. ................................................................. 150
ACKNOWLEDGEMENTS

Firstly, I am deeply grateful to my advisor, Dave Eissenstat, who gave me incredible amounts of advice and feedback and encouraged me to continually improve my work. I am also thankful for my committee members, Eric Post, Margot Kaye, and Kathy Brown, who gave me invaluable guidance and suggestions throughout my time at Penn State. My fieldwork would not have been possible without the help of research assistants and the CPS staff. In particular, Kathy Young and Audrey Mills worked tirelessly to make logistics run smoothly in Greenland, and I would not have been able to conduct this research without them. I am also thankful to friends and family who supported me and kept me laughing. In particular, my State College friends made Penn State a wonderful place to spend four years, and I am grateful for the positivity they brought me. I am especially thankful to my family, particularly Mark, Sheila, and Ben Radville, who gave me unending encouragement, boxes of cupcakes, and hand-painted cards in the mail. Finally, I thank Jason Wittenbach, whose unfailing confidence in me makes me stronger.
Chapter 1

Root Phenology in a Changing Climate

Laura Radville\textsuperscript{1}, M. Luke McCormack\textsuperscript{2,3}, Eric Post\textsuperscript{4}, David M. Eissenstat\textsuperscript{5}

\textsuperscript{1}Department of Ecosystem Science and Management and the Ecology Graduate Program, The Pennsylvania State University, University Park, PA, USA

\textsuperscript{2}Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, China

\textsuperscript{3}Department of Plant Biology, University of Minnesota, St. Paul, MN 55108.

\textsuperscript{4}Department of Biology, The Pennsylvania State University, University Park, PA, USA

\textsuperscript{5}Department of Ecosystem Science and Management and the Ecology Graduate Program, The Pennsylvania State University, University Park, PA, USA

Published: Journal of Experimental Botany (2016), doi:10.1093/jxb/erw062

Abstract

Plant phenology is one of the strongest indicators of ecological responses to climate change, and altered phenology can have pronounced effects on net primary production, species composition in local communities, greenhouse gas fluxes, and ecosystem processes. Although many studies have shown that aboveground plant phenology advances with warmer temperatures, demonstration of a comparable association for belowground phenology has been lacking because the factors that influence root phenology are poorly understood. Because roots can constitute a large fraction of plant biomass, and root phenology may not respond to warming in the same way as shoots, this represents an important knowledge gap in our understanding of how climate...
change will influence phenology and plant performance. We review studies of root phenology and provide suggestions to direct future research. Only 29% of examined studies approached root phenology quantitatively, strongly limiting interpretation of results across studies. Therefore, we suggest that researchers emphasize quantitative analyses in future phenological studies. We suggest that root initiation, peak growth, and root cessation may be under different controls. Root initiation and cessation may be more constrained by soil temperature and the timing of carbon availability, whereas the timing of peak root growth may represent trade-offs among competing plant sinks. Roots likely do not experience winter dormancy in the same way as shoots: 89% of the studies that examined winter phenology found evidence of growth during winter months. More research is needed to observe root phenology, and future studies should be careful to capture winter and early season phenology. This should be done quantitatively, with direct observations of root growth utilizing rhizotrons or minirhizotrons.

**Keywords:** phenology, root growth, root dynamics, belowground, endogenous, exogenous

**Introduction**

One of the most sensitive indicators of a warming climate has been shifts in species phenology, which can have multiple repercussions on ecosystem processes and feedbacks on greenhouse gases (Cleland et al., 2007; IPCC, 2014; Menzel et al., 2006b; Post, 2013; Rosenzweig et al., 2008). The Intergovernmental Panel on Climate Change (IPCC) concluded that phenology is the simplest way to track the ecological impacts of climate change (Solomon et al., 2007), but explicit consideration of belowground phenological responses to climate change has been absent from all five IPCC assessment reports to date. Although air temperature may be the strongest control on aboveground plant phenology (Wielgolaski, 1999), very little is
understood about the drivers of root phenology and how root phenology will respond to climate change.

Many studies examine drivers of root phenology by correlating all increases and decreases in yearly root production with a suite of biotic and abiotic factors. Root phenology may be controlled by different factors throughout the year, and this method may mask differences between the onset, peak, and cessation of root growth. Aboveground, leaf phenology is typically separated into discrete events, such as bud burst and leaf senescence, and these are quantitatively compared with abiotic and biotic factors. Flushes of root growth in summer may be controlled by different factors than the initiation of root growth in the spring. For this review, we conducted a literature search in Web of Science for studies that examined the phrase “root phenology” or “roots” with the terms “growth”, “dynamics”, and “production”. We also included several horticultural publications we were aware of that did not come up in this search. We found 65 published papers that matched these criteria (see Table 2 for list of studies). We focus on studies that used minirhizotrons or rhizotrons, as they allow researchers to nondestructively follow growth of the same roots throughout the year. While minirhizotron tubes and rhizotrons may impact total root production at the transparent wall-soil interface, particularly at shallow depths (Bragg et al., 1983; Ephrath et al., 1999), transparent wall techniques are probably the most accurate way to track the timing of root production (Majdi, 1996). Despite their benefits, only 58% of root phenology studies used rhizotron methods (Table 1). Only 29% (11 of 38 minirhizotron or rhizotron studies) of the studies reviewed here included quantitative evaluation of timing of root growth, such as the date that 10% of roots began growing or the date of first root growth (Table 1). Some studies examined the timing of root initiation, but this was usually done qualitatively.

We advocate for wider and more consistent use of quantitative approaches to characterize multiple aspects of root phenology, such that root initiation, peak root growth, and root cessation
are explicitly defined. For example, root initiation can be quantified as the date at which 50% of minirhizotron tubes or 50% of independent experimental units (when several minirhizotrons are used per experimental unit) contain the first root growth of the season. This perspective is widely established in studies of aboveground phenology, where the percent of plants or plots in the emergent or flowering stages is a common metric (Hoye et al., 2013; Parker et al., 2011; Post et al., 2008; Zadoks, 1985). Furthermore, plants may rely on different cues for the initiation of root phenology in spring, the timing of peak growth, and cessation of root growth in autumn, so consideration of these events separately is warranted.

Finally, we propose that root phenology may have different phenological controls than aboveground phenology, as mounting evidence suggests that onset and progression of root phenology does not simply track aboveground phenology (Abramoff and Finzi, 2015; Blume-Werry et al., 2015). Furthermore, terrestrial biosphere models often include root growth as a fixed, synchronous fraction of leaf photosynthesis, but root growth is often asynchronous with leaf growth (Abramoff and Finzi, 2015). One important difference is that roots, unlike shoots, often do not experience winter dormancy (Fernandez and Caldwell 1975). This may increase interannual variability in root phenology and decrease the likelihood that root and shoot phenology are synchronous. Below, we discuss ways in which environmental controls may differ among phenological events and between above- and belowground organs, evidence for a lack of winter dormancy, and the potential impacts of climate change on belowground phenology.

**Background**

Aboveground plant phenology is a well-studied component of climate change, and temperature may be the most important environmental factor controlling the timing of spring phenology (Wielgolaski, 1999; Wolkovich et al., 2012). For example, spring phenology has
advanced by about 2.5 days per decade in Europe with recent warming (Menzel et al., 2006a).

However, the drivers of aboveground autumn phenology are less clear, but recent studies suggest
that the timing of autumn leaf senescence is linked to the timing of spring budburst, with an
earlier spring linked to earlier autumn leaf senescence (Keenan and Richardson, 2015).

Alternatively, warming may extend aboveground plant growth later into autumn (Marchand et al.,
2004; Natali et al., 2012) or have limited effects on autumn phenology (Kummerow and Russell,
1980; Pudas et al., 2008). Recent experimental evidence indicates that CO₂ fertilization may
further extend the length of the aboveground growing season in response to warming (Reyes-Fox
et al., 2014).

Although the effects of warming on aboveground phenology are fairly well understood,
the effects of warming on belowground phenology have received much less attention. In Figure 1,
we outline potential factors that may be important to root phenology. There is conflicting
evidence regarding the relative influence of exogenous factors, such as soil temperature, and
endogenous factors, such as photosynthate supply, on belowground phenology. In regions with a
distinct winter season, root growth often increases as soil temperatures increase in the spring, and
root growth over winter is limited (Comas et al., 2005; McCormack et al., 2014). Indeed, several
studies have found a positive correlation between soil temperature and root production (Burke
and Raynal, 1994; Steinaker and Wilson, 2008; Steinaker et al., 2010). Other studies, however,
have failed to find a correlation with soil temperature and suggest that endogenous factors, such
as photosynthate availability, are more important to root phenology (Joslin et al., 2001; Sword et
al., 1996; Tierney et al., 2003). The importance of photosynthetic activity was supported by links
of photosynthetic photon flux density (PPFD) to root growth in temperate and upland grasslands
in regions where cloudy days are common (Edwards et al., 2004; Fitter et al., 1999).

Endogenous and exogenous factors likely both play a role in root phenology (Fig. 1, Steinaker
and Wilson, 2008; Tierney et al., 2003), and these roles may differ between species and
environments as plants seek to balance their need to increase their competitiveness with maximizing carbon, water and nutrient use efficiencies. Species-specific differences in phenological strategies are suggested by a common garden experiment in central Pennsylvania where root phenology differed widely between temperate tree species, with some species showing a conservative root phenology and others having high interannual variability in root phenology (McCormack et al., 2014; McCormack et al., 2015). In the common garden, species from varied taxa were planted in the same field in replicated plots so they experienced the same environmental conditions. This suggests that different species may have different strategies to deal with interannual variability in resource supply and climatic constraints. For example, we expect species for which root growth is more cued to shoot growth or to photoperiod would show less spring variability in root initiation than species that respond readily to increases in soil temperature. Moreover, we expect that controls on root growth will change through time, and below we outline the hypothesized influence of external and internal controls throughout the year.

**Initiation of root growth**

Because root growth may not begin below about 5°C in temperate environments (Alvarez-Uria and Koerner, 2007), soil temperature may be a direct control on root initiation. This is supported by the large suppression of winter root growth seen across many ecosystems in seasonal environments (Comas et al., 2005; Tierney et al., 2003). Once soil temperatures are favorable, carbon availability for root production may also constrain the timing of root initiation. The signal to produce new roots is then created by some unknown upregulation of plant growth factors that increase the relative carbon sink strength of roots (Kozlowski, 1992).

Carbon for spring root growth may come from current photosynthate or stored carbohydrates. Carbohydrates, particularly starches, can accumulate in woody tissues in autumn,
and these carbohydrates can fuel early spring root growth in the following year (Najar et al., 2014). Stored carbohydrates may be particularly important in deciduous species that do not have photosynthetic tissues early in the season, as seen in arctic *Betula glandulosa* (Kummerow et al. 1983). Early spring root growth may then be advantageous in regions where high nutrient availability occurs before air temperatures are favorable for leaf growth.

Early spring growth may also be important for resource preemption from competing species. Plants may outcompete neighbors by initiating root growth more quickly and gaining access to limiting resources (Harper, 1977; Harris, 1977). Eissenstat and Caldwell (1988) for example, found that an introduced cold-desert grass was able to compete more effectively with desert shrubs than native grasses by more quickly extracting water from the soil early in the growing season.

**Drivers of peak root growth**

Peak root growth comes at the expense of using carbon for aboveground growth, so peak root growth may represent a trade-off between competing plant sinks (Comas et al., 2005). Because it is costly in terms of carbon, peak root growth may be timed to balance carbohydrate availability with periods of high nutrient and water availability. For example, species that evolved in regions with mid-summer drought may be characterized by adaptations that reduce root growth in mid-summer and peak earlier in spring (Joslin et al., 2001). Plants that are nutrient deficient may not be able to produce as many roots early in the season, but they may increase nutrient uptake by increasing peak root production in later spring and summer (Haynes and Gower, 1995). These increases in root growth to compensate for low nutrient stores may be controlled by plant growth regulators (Lopez-Bucio, 2003). In competition, plants may also advance the timing of
peak root growth in order to preemptively access resources and limit the fitness of neighbors (Dybzinski et al., 2011; Eissenstat and Caldwell, 1988).

Because soils are likely above limiting temperatures by the time peak production occurs, we hypothesize that soil temperature plays a smaller role in determining peak root growth than in root initiation. We also expect that stored carbohydrates play a smaller role in the timing of peak root growth if leaves (and photosynthate production) are already present and current photosynthate is available. In species such as evergreens that potentially photosynthesize year-round, albeit at low rates during winter, or in species with high carbohydrate stores, peak root growth may not be tightly coupled to peak leaf growth and root growth may continue fairly consistently throughout the growing season. In other species, root production may be limited by current photosynthate, and root production may have a large peak after leaf production begins. Furthermore, these contrasting patterns can co-occur within the same ecosystem. For example, Lyr and Hoffman (1967) found that root production peaked in early summer for some species, whereas other species in the same environment grew their roots more uniformly throughout the growing season (Fig. 2). Links between photosynthetically active flux density (PPFD) and root production support the importance of photosynthesis to root phenology and potentially to peak root growth. For example, Edwards et al. (2004) found in British grasslands where cloudy days are common that variation in PPFD was the best predictor of root growth.

**Cessation of root growth: implications for a lack of winter dormancy**

Very little is currently understood about drivers of autumn root phenology. Similar to aboveground production, root growth may slow as temperature (in the soil) and carbohydrate availability decrease. Unlike aboveground production, photoperiod likely does not exert strong control on autumn root phenology. If photoperiod were a strong control, then root growth would
cease at some point in autumn and not begin again until spring. Although root growth may be limited below certain temperatures, we suggest that roots are not normally dormant during winter and growth can occur year-round if conditions become favorable. Of 19 studies that measured winter root growth, 89% detected evidence of winter growth (Table 1). For example, Fernandez and Caldwell (1975) recorded root growth even in near-freezing soils during winter in cold-desert plants, Bauerle et al. (2008) recorded winter root growth in *Vitis* spp. in a Mediterranean climate, and Onipchenko et al. (2009) found snow roots growing into snow packs in an alpine environment. Additionally, snow removal studies lead to soil freezing and cause increased fine root mortality (Tierney et al., 2001), which suggests that these roots were not dormant.

Photoperiod may be a stronger cue in some species with root systems that senesce over winter. For example, Shaver and Billings (1977) found that some arctic graminoid species use photoperiod as a cue for seasonal root cessation. This may be a way they resorb carbohydrates and nutrients in these strongly nutrient-limited systems before the soils freeze and the roots senesce. However, in other arctic shrubs and grasses, there was no evidence that root growth necessarily slows as photoperiod decreases (Fig. 3). Photoperiod may be an important control to some species but not others.

**Potential impacts of climate change**

Given the uncertainty concerning controls on root phenology, it is unclear how climate change will impact the timing of root growth. Altered root phenology could have strong impacts on plant resource acquisition (Nord and Lynch, 2009). A large portion of global carbon is stored in roots (Robinson, 2007), with up to five times higher biomass belowground than aboveground in shrublands, tidal marshes, grasslands, and tundra (Mokany et al., 2006). Aboveground phenology may be a particularly poor indicator of overall ecosystem productivity in regions with
more belowground than aboveground biomass (Blume-Werry et al., 2015). Many studies track aboveground phenology either directly or by remote sensing (e.g. Sitch et al., 2007), but these do not account for changes in root phenology. This increases the uncertainty of predicting carbon fluxes in terrestrial ecosystems with a large fraction of the biomass belowground.

Despite the importance of root production in determining whole-plant responses to climate change, very few studies have directly examined the influence of warming and elevated carbon dioxide concentrations ([CO₂]) on root phenology. In the Arctic, experimental warming caused earlier root growth (Sullivan and Welker, 2005) and increased root production (Sullivan et al., 2008). In a scrub-oak ecosystem, elevated [CO₂] increased fine-root production in spring and autumn, which may be due to increased carbon availability for root production during periods critical to resource acquisition (Brown et al., 2009). Experimental evidence is lacking from other ecosystems, however, and more studies are needed that clearly assess the impacts of warmer air temperatures and elevated [CO₂] on the timing and amount of root production.

We hypothesize that altered precipitation regimes may shift the timing of peak root growth, particularly given evidence that water stress often causes root production to shift to more favorable times of year (Joslin et al., 2000). Because root production can be suppressed during periods of drought (Joslin et al., 2001), we expect peak root production to shift to the wettest times of year in regions with reduced annual precipitation. In regions with increased precipitation, peak root growth may no longer be water-limited and may shift to periods when other factors, such as PPFD, are most available.

The upper soil layer may be more influenced by warming, given that air temperature affects shallow soil temperatures more than deep soils. Therefore, ecosystems with high root biomass near the surface, such as in the Arctic, may be particularly affected by global warming. Elevated [CO₂] have been found to increase deep root production in forested ecosystems (Iversen, 2010), and this may extend phenology of deep roots. We speculate that if elevated [CO₂] causes
more carbon to be shuttled to deep roots, deep roots may have advanced root initiation and delayed root cessation, as long as temperatures are not limiting. The influence of depth on phenology is examined in more detail below.

**The importance of depth**

Root phenology differs by depth in certain ecosystems and may be under different controls (Canham et al., 2012), so we expect that warming, elevated [CO$_2$], and altered precipitation regimes will affect shallow and deep roots differently. Phenology may be shifted later in the season at deeper depths, where soil is slower to warm in the spring and may retain moisture from winter soil recharge. In shrub species in Utah, initiation of root growth began later with increasing soil depth, which may be related to the concurrent seasonal increase in soil temperature at each depth (Fig. 4). For example, in mid-April, soil temperatures at 40cm were 5°C and root production from 30-40cm was 1.1 mm day$^{-1}$cm$^{-1}$, and by late May soil temperatures at the same depth were 14°C and root production was 3.5 mm day$^{-1}$cm$^{-1}$. Deep root growth may also allow the plant to access deep water stores during the driest period of year (Fernandez and Caldwell, 1975). Similarly, shallow roots in phreatophytic *Banksia* spp. grow in a pulse, synchronously with aboveground tissues, but deeper roots grow constantly throughout the year, as they have access to a constant deep water source (Canham et al., 2012). Deep root growth may also be timed to use deep water storage during times of water stress, reducing the impact of seasonal droughts (Hendrick and Pregitzer, 1996).

The phenology of shallow roots may be more strongly influenced by environmental factors, such as soil moisture and temperature, than deeper roots (Hendrick and Pregitzer, 1997). For example, in loblolly pine (*Pinus taeda* L.) in southeastern United States, a mid-summer drought reduced new root growth in shallow soil layers, but root initiation continued in deeper
soils (Sword et al., 1996). However, root phenology may not always differ by soil depth (Fig. 4). Despite strong seasonal changes in soil temperature with depth, root phenology in southwestern Greenland was not significantly different across soil depths, even if most roots were produced close to the soil surface (Radville and Eissenstat, unpublished data).

**Future directions and conclusions**

Future studies can more accurately tease apart the controls on phenology if they focus on the explicit timing of events. Use of minirhizotrons or rhizotrons is likely the most accurate way to do this, because destructive methods, such as soil coring, do not follow the same roots through time and will often miss the precise occurrence of new root growth. Because it is unlikely that roots are truly dormant in winter and because roots do not necessarily track aboveground growth, root measurements should begin very early in the season or persist through winter months when feasible. Some previous studies may have missed the onset of root growth if measurements were taken too late in the season or were not taken often enough throughout the year. For example, one study found that root growth preceded leaf growth (McCormack et al., 2015), but another study with less temporally resolved observations using the same plants found root production to peak after major leaf expansion (McCormack et al., 2014). Future studies that continue observations throughout the winter or begin root measurements prior to leaf emergence, that do not assume root phenology tracks aboveground phenology, and that quantitatively evaluate phenology, will enjoy greater success at teasing apart the controls on root phenology. Given that root and shoot growth may respond differently to climate change, this is essential in determining the future impacts of warming on whole-plant carbon use. Because root and shoot phenology often are not synchronous and may not be influenced by warming in the same way, root phenology research is critical to understanding shifts in whole-plant growth with climate change.
Acknowledgments: LR, EP, and DME were supported by U.S. National Science Foundation, Arctic Natural Sciences Program (ARC-110738), and MLM was supported by research fellowships from the Chinese Academy of Sciences and the National Natural Sciences Foundation of China (NSFC) for Young International Researchers (no. 31350110503). We thank two anonymous reviewers for helpful comments on an earlier version of this manuscript.

Figure 1-1: Potential controls over root phenology. Solid lines indicate direct controls and dashed lines indicate indirect controls on root phenology. Gray boxes represent endogenous controls; white boxes represent exogenous controls. PPFD is photosynthetically active flux density.
Figure 1-2: Seasonal shoot and root growth in two deciduous species and two evergreen species in the Eberswalde root laboratory in Germany. Shoot growth is indicated by the dotted area and root growth by the solid area. Figure is adapted from Lyr and Hoffman (1967).

Figure 1-3: Root elongation at different photoperiods. A. Average root elongation (mm day$^{-1}$) at decreasing day lengths from July to September in mixed vegetation plots in Kangerlussuaq, Greenland. Error bars indicate standard error of the mean (n=24 at all day lengths). B. Average root elongation (mm day$^{-1}$) of Dupontia fischeri at decreasing day lengths in Barrow, Alaska, U.S.A. Photoperiod was experimentally controlled in phytotrons. Error bars indicate standard
error of the mean (n=120 at 24h, n=24 at 21h, and n=6 at 18h). Figure adapted from Shaver and Billings (1977).

Figure 1-4: Seasonal root production at three 10 cm depth intervals. A. Average daily root production (mm day$^{-1}$ standardized to a 50 cm$^2$ observation window) in mixed vegetation plots in the Arctic near Kangerlussuaq, Greenland. The timing of root production was not significantly different between soil depths, but soil temperature was significantly different between soil depths. B. Average daily root production (mm day$^{-1}$ in 50 cm$^2$ observation window) in Artemisia tridentata in Curlew Valley, Utah, U.S.A. Adapted from Fernandez and Caldwell (1975).
Table 1-1: The number of studies that quantify the timing of events and found evidence of winter root growth. Only studies that used minirhizotron or rhizotron methods were examined for winter root growth measurements and quantification of root phenology.

<table>
<thead>
<tr>
<th>Percent of papers</th>
<th>Number of papers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minirhizotron or rhizotron methods</td>
<td>58</td>
</tr>
<tr>
<td>Quantified timing of events</td>
<td>29</td>
</tr>
<tr>
<td>Measured winter growth</td>
<td>50</td>
</tr>
<tr>
<td>Evidence of winter root growth</td>
<td>89</td>
</tr>
</tbody>
</table>
Table 1-2: List of literature reviewed with citation, location, climate, vegetation type, species, whether study used minirhizotron or rhizotron methods, whether study was quantitative or qualitative, and whether study found evidence of winter root growth.

* Climate classifications based on reference of study location to standard climate classification scheme (Peel et al., 2007)
1 M = Minirhizotron or rhizotron; O = Other methods
2 Yes = Quantitative; No = Qualitative
3 Yes = Measured winter root growth; No = No winter measurements
4 Yes = Evidence for winter root growth; No = No evidence of winter growth; - = Not applicable (e.g. due to lack of measurements)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location (climate*)</th>
<th>Vegetation</th>
<th>Species</th>
<th>Method1</th>
<th>Quantitative2</th>
<th>Winter data3</th>
<th>Winter growth4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevington and Castle</td>
<td>Florida, USA (Temperate; hot summer)</td>
<td>Tree orchard, irrigated</td>
<td><em>Citrus sinensis</em></td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Blume-Werry et al.</td>
<td>Låktatjåkka, Sweden (Cold; cold summer)</td>
<td>Forest, tundra</td>
<td>Sub-alpine mountain birch forest, low alpine tundra, high alpine tundra</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Bonomelli et al.</td>
<td>Central Chile (Temperate; dry warm summer)</td>
<td>Tree orchard, irrigated</td>
<td><em>Prunus avium</em></td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Authors</td>
<td>Location</td>
<td>Type</td>
<td>Species</td>
<td>M</td>
<td>N</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>--------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>---</td>
<td>---</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td>Broschat (1998)</td>
<td>Florida, USA (Tropical; monsoon)</td>
<td>Tree</td>
<td><em>Roystonea regia</em>, <em>Cocos nucifera</em> ‘Malayan Dwarf’, <em>Syagrus romanzoffiana</em>, <em>Phoenix roebelenii</em></td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Burke and Raynal (1994)</td>
<td>New York, USA (Cold; warm summer)</td>
<td>Tree</td>
<td>Northern hardwood forest dominated by <em>Acer saccharum</em>, <em>Fagus grandifolia</em>, <em>Betula alleghaniensis</em>, <em>Acer rubrum</em></td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Comas <em>et al.</em> (2005)</td>
<td>New York, USA (Cold; warm summer)</td>
<td>Vine, with and without irrigation</td>
<td><em>Vitis labruscana</em> cv. Concord</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Contador <em>et al.</em> (2015)</td>
<td>California, USA (Temperate; dry, hot summer)</td>
<td>Tree orchard, irrigated</td>
<td><em>Juglans regia</em> ‘Chandler’</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Curtis <em>et al.</em> (1994)</td>
<td>Michigan, USA (Cold; warm summer)</td>
<td>Tree</td>
<td><em>Populus grandidentata</em></td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Plant Type</td>
<td>Primary Dominant Species</td>
<td>M</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------------------------------------------</td>
<td>------------</td>
<td>-----------------------------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Edwards <em>et al.</em> (2004)</td>
<td>York, UK (Temperate; warm summer)</td>
<td>Grass</td>
<td>Temperate grassland dominated by <em>Holcus lanatus</em></td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Eissenstat <em>et al.</em> (2006)</td>
<td>New York, USA (Cold; warm summer); California, USA (Temperate; dry, warm summer)</td>
<td>Vine</td>
<td><em>Vitis lambriscana</em>, <em>Vitis vinifera</em>, and <em>Malus × domestica</em></td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fitter <em>et al.</em> (1999)</td>
<td>Cumbria, UK (Temperate; warm summer)</td>
<td>Grass</td>
<td>Grassland dominated by <em>Festuca ovina</em> and <em>Agrostis capillaris</em></td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Harris (1977)</td>
<td>Washington, USA (Cold; dry hot summer)</td>
<td>Grass</td>
<td>Semiarid rangeland, with <em>Agropyron spicatum</em>, <em>Poa sandbergii</em>, <em>Artemisia tridentate</em>, <em>Agropyron spicatum</em></td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Type</td>
<td>Trees</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------</td>
<td>-------------</td>
<td>----------------------------------------------------------------------</td>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Harris et al. (1995)</td>
<td>New York, USA (Cold; warm summer)</td>
<td>Tree</td>
<td>Fraxinus pennsylvanica, Quercus coccinea, Corylus columna, and Syringa reticulata ‘Ivory Silk’</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Head (1968)</td>
<td>Kent, England (Temperate; warm summer)</td>
<td>Tree</td>
<td>Cydonia oblonga</td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hendrick and Pregitzer (1996)</td>
<td>Michigan, USA (Cold; warm summer)</td>
<td>Forest</td>
<td>Northern hardwoods forests dominated by Acer saccharum</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hendrick and Pregitzer (1997)</td>
<td>Michigan, USA (Cold; warm summer)</td>
<td>Forest</td>
<td>Northern hardwoods forests dominated by Acer saccharum</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Ibacache et al. (1999)</td>
<td>La Serena, Chile (Arid; cold desert)</td>
<td>Tree, irrigated</td>
<td>Annona cherimola</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Janos et al. (2008)</td>
<td>Darwin, Australia (Tropical savannah)</td>
<td>Savannah</td>
<td>Eucalyptus tetrodonta savanna</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Joslin et al. (2001)</td>
<td>Tennessee, USA (Temperate; hot summer)</td>
<td>Forest</td>
<td>Quercus alba-Quercus prinus forest</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Vegetation Types</td>
<td>Species</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------------------------------</td>
<td>------------------------------------------------------</td>
<td>----------------------------------------------</td>
<td>---</td>
<td>----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Marler and Willis (1996)</td>
<td>Florida, USA (Tropical monsoon)</td>
<td>Tree, irrigated</td>
<td><em>Litchi chinensis</em></td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>McCormack et al. (2010)</td>
<td>Alabama, USA (Temperate; hot summer)</td>
<td>Tree, grass, forb; irrigated</td>
<td><em>Pinus palustris,</em> <em>Quercus margaretta,</em> <em>Aristida stricta,</em> <em>Crotalaria rotundifolia,</em> <em>Asclepias tuberosa</em></td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>McCormack et al. (2014)</td>
<td>Pennsylvania, USA (Cold; warm summer)</td>
<td>Tree</td>
<td><em>Acer negundo,</em> <em>Acer rubrum,</em> <em>Acer saccharum,</em> <em>Carya glabra,</em> <em>Juglans nigra,</em> <em>Liriodendron tulipifera,</em> <em>Pinus strobus,</em> <em>Pinus virginiana,</em> <em>Populus tremuloides,</em> <em>Quercus alba,</em> <em>Quercus rubra,</em> <em>Sassafras albidum</em></td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Authors</td>
<td>Location</td>
<td>Study Type</td>
<td>Species</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------------</td>
<td>------------</td>
<td>--------------------------------------------------------------------------</td>
<td>---</td>
<td>-----</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>McCormack et al. (2015)</td>
<td>Pennsylvania, USA (Cold; warm summer)</td>
<td>Tree</td>
<td>Acer negundo, Acer rubrum, Acer saccharum, Carya glabra, Juglans nigra, Liriodendron tulipifera, Pinus strobus, Pinus virginiana, Populus tremuloides, Quercus alba, Quercus rubra, Sassafras albidum</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Mickelbart et al. (2012)</td>
<td>California, USA (Arid; cold steppe)</td>
<td>Tree</td>
<td>Persea americana</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Misson et al. (2006)</td>
<td>California, USA (Arid; cold steppe)</td>
<td>Tree plantation</td>
<td>Pinus ponderosa dominated plantation</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Nomura and Kikuzawa (2003)</td>
<td>Sabah, East Malaysia (Tropical rainforest)</td>
<td>Forest</td>
<td>Lower montane forest, upper montane forest, subalpine forest</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Plantation Type</td>
<td>Species/Description</td>
<td>Growth Form</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>----------------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>--------------</td>
<td>---</td>
<td>----</td>
<td>-----</td>
</tr>
<tr>
<td>Ploetz et al. (1992)</td>
<td>Florida, USA (Tropical savannah)</td>
<td>Tree, irrigated</td>
<td>Persea americana</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>(Pritchard et al., 2008)</td>
<td>North Carolina, USA (Temperate; hot summer)</td>
<td>Tree</td>
<td>Pinus taeda</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Psarras et al. (2000)</td>
<td>New York, USA (Cold; warm summer)</td>
<td>Tree, irrigated</td>
<td>Malus sylvestris var. domestica</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Quan et al. (2010)</td>
<td>Northeastern China (Cold; dry winter; warm summer)</td>
<td>Forest, plantation</td>
<td>Mongolian oak forest, aspen-birch forest, hardwood forest, Korean pine plantation, Dahurian larch plantation</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Reich et al. (1980)</td>
<td>Missouri, USA (Temperate; hot summer)</td>
<td>Tree, irrigated</td>
<td>Quercus alba, Q. marilandica, Q. velutina</td>
<td>M</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Repo et al. (2008)</td>
<td>Finland (Cold; cold summer)</td>
<td>Tree, irrigated</td>
<td>Pinus sylvestris</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Rytter (2001)</td>
<td>Uppsala, Sweden (Cold; warm summer)</td>
<td>Shrub, irrigated</td>
<td>Salix viminalis</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Steinaker and Wilson (2008)</td>
<td>Saskatchewan, Canada (Cold; warm summer)</td>
<td>Forest, grassland</td>
<td>Native grassland and aspen forest (Populus tremuloides)</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Type</td>
<td>Dominant Species</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------------------------------</td>
<td>-----------</td>
<td>----------------------------------------------------------------------------------</td>
<td>----</td>
<td>-----</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>Steinaker et al. (2010)</td>
<td>Saskatchewan, Canada (Cold; warm summer)</td>
<td>Tree, shrub, grass</td>
<td><em>Festuca rubra, Koeleria gracilis, Poa compressa, Bouteloua gracilis, Schizachyrium scoparium, Rosa woodsii, Shepherdia canadensis, Symphoricarpos occidentalis, Prunus virginiana, Picea glauca</em></td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Sword et al. (1996)</td>
<td>Louisiana, USA (Temperate; hot summer)</td>
<td>Tree</td>
<td><em>Pinus taeda</em></td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Tierney et al. (2003)</td>
<td>New Hampshire, USA (Cold; warm summer)</td>
<td>Forest</td>
<td>Dominated by <em>Fagus grandifolia, Acer saccharum, and Betula alleghaniensis</em></td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Wan et al. (2004)</td>
<td>Tennessee, USA (Temperate; hot summer)</td>
<td>Tree</td>
<td><em>Acer rubrum, Acer saccharum</em></td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Authors</td>
<td>Location</td>
<td>Type</td>
<td>Species</td>
<td>Season</td>
<td>Irrigation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------------------</td>
<td>------------</td>
<td>----------------------------------</td>
<td>--------------</td>
<td>------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerts et al. (1992)</td>
<td>Utrecht, Netherlands (Temperate; warm summer)</td>
<td>Sedge</td>
<td>Carex diandra, C. rostrata, C. lasiocarpa</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Barnes (2002)</td>
<td>Alabama, USA (Temperate; hot summer)</td>
<td>Tree, irrigated</td>
<td>Pinus taeda</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bosac et al. (1995)</td>
<td>England, UK (Temperate; warm summer)</td>
<td>Tree, irrigated</td>
<td>Populus euramericana ‘Primo’</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Burke et al. (1992)</td>
<td>New York, USA (Cold; warm summer)</td>
<td>Tree, irrigated</td>
<td>Acer saccharum</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Canham et al. (2012)</td>
<td>Western Australia, Australia (Temperate; dry, hot summer)</td>
<td>Forest</td>
<td>Banksia attenuata, Banksia ilicifolia</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardon et al. (2002)</td>
<td>Connecticut, USA (Cold; hot summer)</td>
<td>Tree, with and without irrigation</td>
<td>Quercus rubra</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coll et al. (2012)</td>
<td>Agüero, Spain (Cold; warm summer)</td>
<td>Forest</td>
<td>Quercus ilex, Quercus faginea</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Côté et al. (1998)</td>
<td>Québec, Canada (Cold; warm summer)</td>
<td>Forest</td>
<td>Forest dominated by Acer saccharum</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dornbush and Raich (2006)</td>
<td>Iowa, USA (Cold; hot summer)</td>
<td>Grass</td>
<td>Cool-season meadow and warm-season meadow</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Drew and Ledig (1981)</td>
<td>North Carolina, USA (Temperate; hot summer)</td>
<td>Tree</td>
<td>Pinus taeda</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Location</td>
<td>Type</td>
<td>Species/Forest Type</td>
<td>Notes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
<td>------</td>
<td>---------------------</td>
<td>-------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Du and Fang (2014)</td>
<td>Great Khingan Mountains, China (Cold; dry winter; warm summer)</td>
<td>Forest</td>
<td><em>Betula platyphylla</em> – <em>Populus davidiana</em> forest; <em>Larix gmelinii</em> forest</td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dušek and Kvet (2006)</td>
<td>South Bohemia, Czech Republic (Cold; warm summer)</td>
<td>Shrub</td>
<td><em>Salix caprea</em></td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haynes and Gower (1995)</td>
<td>Wisconsin, USA (Cold; warm summer)</td>
<td>Tree plantation</td>
<td><em>Pinus resinosa</em></td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hester et al. (2004)</td>
<td>Scotland, UK (Temperate; warm summer)</td>
<td>Tree, irrigated</td>
<td><em>Betula pendula</em>, <em>Pinus sylvestris</em>, <em>Sorbus aucuparia</em></td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaakinen et al. (2004)</td>
<td>Northern Savonia, Finland (Cold; cold summer)</td>
<td>Tree, irrigated</td>
<td><em>Picea abies</em></td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaur et al. (2012)</td>
<td>Nova Scotia, Canada (Cold; warm summer)</td>
<td>Shrub</td>
<td><em>Vaccinium angustifolium</em></td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kho et al. (2013)</td>
<td>Sarawak, Malaysia (Tropical rainforest)</td>
<td>Forest</td>
<td>Lowland mixed dipterocarp forest</td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Konopka et al. (2005)</td>
<td>Brasschaat, Belgium (Temperate; warm summer)</td>
<td>Forest</td>
<td><em>Quercus robur</em>, <em>Pinus sylvestris</em></td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lahti et al. (2005)</td>
<td>North Karelia, Finland (Cold; cold summer)</td>
<td>Tree, irrigated</td>
<td><em>Picea abies</em></td>
<td>O - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Location</td>
<td>Dominance</td>
<td>Species Presence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palacio and Montserrat-Marti (2007)</td>
<td>Jaca, Zaragoza, and Villamayor, Spain (Cold; warm summer, Arid; cold steppe)</td>
<td>Shrub, forb</td>
<td>Echinopsartum horridum, Salvia lavandulifolia, Lepidium subulatum, Linum suffruticosum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papatheodorou et al. (1998)</td>
<td>Greek Macedonia, Greece (Temperate; hot summer)</td>
<td>Shrub</td>
<td>Quercus coccifera</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson and Puttonen (1991)</td>
<td>Helsinki, Finland (Cold; warm summer)</td>
<td></td>
<td>Pinus sylvestris, Picea abies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tingey et al. (1996)</td>
<td>California, USA (Temperate; dry, hot summer)</td>
<td>Tree, irrigated</td>
<td>Pinus ponderosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vapaavuori et al. (1992)</td>
<td>Northern Savonia, Finland (Cold; cold summer)</td>
<td>Tree, irrigated</td>
<td>Pinus sylvestris, Picea abies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yamashita and Imamura (2007)</td>
<td>Fukuoka, Japan (Temperate; hot summer)</td>
<td>Tree, irrigated</td>
<td>Eustoma grandiflorum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zasada et al. (1994)</td>
<td>Oregon, USA (Temperate; dry, warm summer)</td>
<td>Shrub, irrigated</td>
<td>Rubus spectabilis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
References


Sword MA, Gravatt DA, Faulkner PL, Chambers JL. 1996. Seasonal branch and fine root growth of juvenile loblolly pine five growing seasons after fertilization. Tree Physiology 16, 899-904.


Chapter 2

**Limited linkages of above- with belowground phenology: A study in grape**

Laura Radville\(^2\), Taryn L. Bauerle\(^{2,3}\), Louise H. Comas\(^{2,4}\), Katherine A. Marchetto\(^2\), Alan N. Lakso\(^5\), David R. Smart\(^6\), Richard M. Dunst\(^5\), David M. Eissenstat\(^2\)

\(^2\)Department of Ecosystem Science and Management and the Ecology Graduate Program, The Pennsylvania State University, State College, PA, USA; \(^3\)School of Integrative Plant Science, Cornell University, Ithaca, NY, USA; \(^4\)USDA-ARS Water Management and Systems Research Unit, Fort Collins, CO, USA; \(^5\)Horticulture Section, Cornell University, New York State Agricultural Experiment Station, Geneva, NY, USA; \(^6\)Department of Viticulture and Enology, University of California- Davis, Davis, CA, USA

Published in *American Journal of Botany*, November 2016

**Abstract**

*Premise of the study*: Plant phenology impacts resource utilization, carbon fluxes, and interspecific interactions. Although controls on aboveground phenology have been studied to some degree, controls on root phenology are exceptionally poorly understood.

*Methods*: We used minirhizotrons to examine the timing of grape root production over five years in Fredonia, New York under a humid, continental climate, and over three years in Oakville, California, under a Mediterranean-type climate. We used data from previous experiments to examine the relationship of root phenology with aboveground phenology. We compared interannual variability in root and shoot growth and determined the influence of abiotic factors on
the timing of root initiation, peak root standing crop, peak root growth rate, and cessation of root growth.

**Key results:** Root phenology was not tightly coupled with aboveground phenological periods. Both sites typically had one yearly root flush and high interannual variability in root growth. Root phenology in California was more variable than in New York. In this study and other published studies, interannual variation in root phenology was greater than variation in aboveground phenology. The three phenological phases of root growth, root initiation, peak root growth, and root cessation, were related to different suites of abiotic factors.

**Conclusions:** Root phenology is highly variable among years. Analysis of potential controlling factors over several years suggest that belowground phenological phases should be analyzed separately from each other. If aboveground grape phenology responds differently than belowground phenology to changes in air temperature, global warming may further uncouple the timing of above- and belowground growth.

**Keywords:** ‘Concord’; ‘Merlot’; minirhizotrons; root growth; root phenology; *Vitis labruscana*; *Vitis vinifera*

**Introduction**

The timing of root growth has a strong influence on water and nutrient acquisition, whole-plant growth and carbon fluxes, plant competition, and interactions with soil symbionts, pathogens, and herbivores (Bauerle et al., 2007; Eissenstat and Caldwell, 1988; Resendes et al., 2008; Volder et al., 2005). Substantial work has been conducted on the periodicity of aboveground tissues, including shifts in plant phenology with climatic change (Crepinsek et al., 2012; Fitter and Fitter, 2002; Post et al., 2008) and timing of shoot flushing (Alaoui-Sosse et al.,
1994; Alatou et al., 1989; Reich et al., 1980). Much less is known about the growth phenology of roots, including the influence of climate change (Abramoff and Finzi, 2015; McCormack et al., 2015). Climate change may alter the timing and duration of root growth, which could impair resource acquisition if root growth no longer coincides with peak resource availability (Nord and Lynch, 2009).

The timing of first root growth in spring, peak root growth and peak root standing crop in summer, and root cessation in fall are ecologically important but poorly understood. There is some evidence that timing of root growth is synchronized with shoot growth with several processes potentially contributing control that could couple or uncouple the synchronization (Comas et al., 2005). The timing and amount of root growth may be controlled by endogenous factors associated with carbohydrate supply and competing resource sinks, and exogenous factors, such as temperature, availability of nutrients, and soil moisture, with the relative influence of endogenous and exogenous factors still unclear (Comas et al., 2005; Rojas-Jimenez et al., 2007; Sword et al., 1996). The timing of root production may also be influenced by upregulation of plant growth factors (Kozlowski, 1992). In hardwood forests in New York, Burke and Raynal (1994) found a predictable relationship between fine root growth and soil temperature alone. Other studies, however, found no correlation between root growth and soil temperature and suggest that endogenous factors, such as photosynthate availability, are more important controls on root growth (Joslin et al., 2001).

Factors controlling periods of abundant root growth (root flushes) during the growing season are also unclear. Early work with fruit crops using large rhizotrons indicated that two root flushes occur on a yearly basis: one in the spring and one in the fall. This seasonal pattern may be caused by competition with developing reproductive organs and has been noted in textbooks (apple: Fallahi, 1994; grape: Mullins et al., 1992; apple: Rom, 1996). Endogenous source:sink controls of root growth have been attributed to root competition with reproductive growth and
shoot growth in several studies (*Prunus persica*: Berman and Dejong, 2003; *Vitis labruscana*: Comas et al., 2005; *Juglans regia*: Contador et al., 2015; *Quercus prinus, Quercus alba, Cornus florida*, and *Acer rubrum*: Joslin et al., 2001; *Quercus alba*: Reich et al., 1980; *Hevea brasiliensis*: Thaler and Pages, 1996). Additionally, in fruit crops that have been selected for high reproductive growth, growth of reproductive organs may reduce stored carbohydrate reserves, affecting root growth in the early part of the following year (Comas et al., 2005). Alternatively, mid-season declines in root growth may be controlled by exogenous factors. During the summer in temperate regions, high soil temperature (Contador et al., 2015; Kaspar and Bland, 1992), limited water availability (King et al., 1999; Tierney et al., 2003), and low photosynthetically active radiation (such as by shading, Edwards et al., 2004) may limit root growth. In winter, low soil and air temperatures and low or no photosynthesis are generally assumed to limit root growth.

The lack of long-term datasets may be one reason for conflicting hypotheses about the controls on root phenology and the degree to which above- and belowground phenology are linked. Many studies attempt to make conclusions based on only two years of data (Côté et al., 1998; Edwards et al., 2004; Tierney et al., 2003). When there is high year-to-year variation in seasonal root production patterns, limited seasons of observation may constrain attempts to discern general patterns.

In order to overcome these limitations, we examined unique minirhizotron datasets of three to five years of grape plants. Grape is an ideal system in which to compare above- and belowground phenology because growers keep detailed records of aboveground phenological events as part of standard viticultural practices for timing of pesticide sprays and in predicting timing of fruit maturation. Our objectives were to examine whether belowground phenology can be broadly estimated from aboveground phenology and to examine the timing of root initiation, peak root standing crop, maximum root growth rate, and root cessation in grapes grown in
Fredonia, New York under a humid continental climate (a cold winter and a warm summer without a dry season) and in Oakville, CA under a Mediterranean climate (a temperate dry climate with cool winters and warm summers) (Köppen-Geiger climate classification system, Peel et al., 2007). We examined the influence of abiotic factors on the timing of belowground growth and conducted a literature review to compare above- and belowground variability in other published studies. We predicted that periods of belowground growth broadly track the timing of aboveground growth. We also expected that the timing of root initiation, peak root growth, and root cessation are driven by different abiotic factors and that belowground phenology is more variable than aboveground phenology.

**Methods**

*Humid Continental study site and plant material*

*Vitis labruscana* Bailey cv. Concord vines were grown at Cornell University’s Vineyard Laboratory in Fredonia, New York (NY), USA. Soils in this location were well-drained Chenango gravelly loam at least 2 m deep with no restrictive layers. Concord vines were trained to a high-wire bilateral cordon system located 1.8 m above the ground, spaced 2.4 m between vines and 2.7 m between rows.

Vines were grown in a two by two factorial design of pruning (minimal or heavy pruning) and irrigation (with and without irrigation). There was a buffer vine on each end of the experimental unit that was not measured. The row orientation was SW-NE and vines were 40+ years old. Heavy pruning consisted of pruning the vines to 44 buds per kg of pruned canes (dormant shoots) in the winter while minimally pruned vines were hedge undercut to 1 meter to keep the vegetation off of the ground, and there was also a vine separation cut. Irrigated vines received supplemental drip irrigation as needed to maintain soil moisture adequate for canopy
growth and full ripening of the crop. Each experimental unit represented five vines each in four replicate plots. Each experimental unit had four minirhizotron tubes for a total of 16 tubes per treatment. We took the sum of all root growth visible on the outside of each tube and then averaged root growth data from the four tubes in each unit.

Minirhizotron tubes (cellulose acetate butyrate) were installed in the fall of 1996 at 30 degrees from vertical, perpendicularly to vine rows and 0.5 m from vine trunks (see Anderson et al., 2003 for details). Tubes were 183 cm long with an outside diameter of 5.7 cm and marked with 127 numbered observation windows that were 1.5 cm tall and 1.0 cm wide. Root images were collected with a small video camera (Bartz Technology, Santa Barbara, CA) through marked observation windows scribed onto minirhizotron tubes. We recognize that tube material can affect root dynamics in some species (Withington et al., 2003); however, all comparisons are made with the same tube material in a single species and we do not expect interactions of tube material and time of year. From 1998 to 2002, root images were captured on S video an average of every two weeks during the growing season and less frequently during the fall and winter. Root length was digitized for all images on all dates by saving stills from the videos and manually sketching roots on paper. Data were entered into a digital spreadsheet to track root counts per window and dates of changes and to convert root numbers to root length.

The relative amount of root growth was calculated for each observation date by dividing the length of roots produced on each sampling date by the total length of roots produced that year for that tube. Root production was defined as the appearance of new roots, while standing root crop is the total number of roots present (live roots minus dead roots; i.e., cumulative root production - cumulative root mortality). The total relative amount of root growth was then separated by the aboveground phenophases, and the average (±SE) relative amount of root growth in each time period was calculated across all years. The aboveground stages were dormancy to bloom (January to mid-June), bloom to veraison (mid-to-late August when fruit begins to ripen
and red varieties turn from green to red), veraison to harvest (early October), and harvest to leaf fall (late November). Bloom, veraison, and harvest were chosen because they are metrics that are typically recorded by growers, and in this study the date of each event was collected each year. These data are included in Appendix S1 (see Supplemental Data with the online version of this article). For the following metrics and in figures, experimental units that had fewer than 20 roots or fewer than 20 root births throughout the year were excluded. This was done to avoid inaccuracies in percentile estimates or timing estimates caused by small root population sizes.

Root initiation was recorded as the date at which the first root appeared in a given experimental unit (n=4 minirhizotron tubes per experimental unit). The date at which 50% of the experimental units (with greater than 20 roots) showed any amount of root growth was recorded as an estimate of seasonal root growth initiation. This metric was selected to compare to the typical method of reporting aboveground phenological stages in grape. These stages are defined by the dates of development, such as the dates of budbreak or initial growth, flowering, veraison (starting of ripening), and harvest (Parker et al., 2011). The date of peak root standing crop (m m⁻²) was calculated as the date following a two-week sampling interval that an experimental unit had the maximum amount of roots present for the year. The date at which 50% of experimental units had reached peak standing crop was used as a seasonal estimate of peak root standing crop. The date of highest root production was recorded as the date with the largest increase in extension of new roots since the previous sampling date (m m⁻² day). The date of root cessation was calculated as the date at which at least three sampling dates in a row had less than 5% of total root production for the season. Solar radiation data came from the Earth System Research Laboratory’s Surfrad program (http://www.esrl.noaa.gov/gmd/grad/surfrad/).

The data collected from 1997-2000 were used by Anderson et al. (2003) to examine root lifespan and by Comas et al. (2005) to examine factors controlling root dynamics. Data collected in 1998, 2000, and 2002 in the balanced pruned treatment in New York and data from 2003 in the
California vines were previously used in a symposium review paper (Eissenstat et al. 2006). This paper had limited analyses regarding phenology and did not include the entire dataset evaluated here. Data collected in 1997 were omitted in the present study due to a lag in spring growth that followed the installation of the minirhizotron tubes.

*Mediterranean-type climate study site and plant material*

The experiment was set up in an established Merlot (*Vitis vinifera* cv. Merlot) experimental block in Oakville, California (CA), USA and managed in cooperation with UC Davis. The vines were 11 years old, grafted on 1103P and 101-14 Mgt rootstocks. Soil was a Bale (variant) gravelly loam, a fine-loamy, mixed, superactive, thermic Cumulic Ultic Haploxeroll. The vines were trained on a bilateral cordon with vertical shoot positioning (VSP) and oriented SE to NW with rows of vines respectively spaced 2.4 m between vines and 2.2 m between rows.

Minirhizotron root observation tubes (1.3 m in length and 6 cm in outside diameter, cellulose acetyl butyrate plastic) were installed in April 2002 at an angle of 30˚ from the vertical to a vertical depth of 1.1 m of which 1 m was accessible using the minirhizotron camera. Two minirhizotron tubes were installed per vine with three vines per plot and six replicate blocks. Each of the three vines in a plot was assigned to a different irrigation treatment, but only unirrigated control vines were used in this paper. Minirhizotron images were taken from January 2003 to December 2005, once every two weeks during the growing season and once a month during vine dormancy (see Bauerle et al., 2008 for more details). The relative amount of new root growth and the proportion of root growth in each broad time period (January to bloom, bloom to veraison, veraison to harvest, and harvest to November) were calculated in the same way as the NY dataset. Dates of aboveground phenological stages were recorded in each year. Images were analyzed using Win Rhizo Tron MF software (Regents Inc. Quebec, Canada) for root seasonal production.
The data collected from this site were used by Bauerle et al. (2008) to examine the influence of plant vigor and soil moisture stress on root survival.

Comparing above- and belowground phenology from literature

In order to place these results in a broader context, Web of Science was used to search the literature for all studies examining “root phenology”, “root dynamics”, and “belowground phenology”. This review differs from Abramoff and Finzi (2015) because we only included studies that directly tracked timing of root growth using minirhizotron methods (as opposed to other destructive or indirect measures) and took measurements across at least two years. We excluded one study, Comas et al. (2005), because it drew from the same dataset as the present study. In each study, the timing of peak root growth and either the timing of peak aboveground (leaf or stem) growth or date of bloom (if leaf or stem growth was not available) were extracted from the published figures. While it would be interesting to separate vegetative from reproductive growth, we already had very few studies that met our criteria, so we were not able to make comparisons among categories of aboveground growth. This deserves attention in future studies, however, because drivers of leaf, stem, and fruit phenology have been shown to differ from each other (for example, Post et al., 2008). The date of peak leaf or stem growth and the date of bloom are important to belowground phenology because they represent the timing of carbon production and competing carbon sinks. In order to compare interannual variability in above- and belowground phenology, the coefficient of variation (C.V.) of dates of peak shoot and root growth among years was calculated separately for each study. For studies that used multiple species or multiple soil types, the C.V.s were calculated for each species or soil type and then averaged for the entire study. We used the closest meteorological station from NOAA (National Oceanic and Atmospheric Administration) National Centers for Environmental Information, or when data were not available there, from the Weather Underground
(http://www.wunderground.com/) to obtain growing degree days (GDD; base 10°C) for all dates of peak root and shoot growth and computed C.V.s for GDDs.

_Estimating fine-scale root phenology with degree days_

Multiple types of degree-day measurements were calculated to determine which method best fit the data for subsequent analysis. Degree-days, degree-days subtracting chilling below 0°C, and degree-days using a $Q_{10}$ of 2 were calculated with critical temperatures for initiating growth of 8, 10, and 12°C. These were compared to the root growth data by calculating coefficients of variation for different stages of new root production and phenology: rooting initiation, 5% root production, 10% root production, 15-25% root production, and bloom date. The standard degree-day model that fit the data best used critical temperatures of 8°C and 10°C. The latter temperature (10°C) was chosen to match the critical temperature used for pest management and other horticultural practices (Lake Erie Regional Grape Program, 2013).

_Statistical analyses_

Root growth data from the four tubes in each experimental unit were averaged for all statistical analyses. Interannual variation in the proportion of root growth during each aboveground phenophase was examined with ANOVAs. For each aboveground phenophase (January to bloom, bloom to veraison, veraison to harvest, and harvest to November), year was used as the independent variable and proportion of root growth was used as the dependent variable. To test whether the variance in the proportion of root growth in each aboveground phenophase was equal between the NY and CA sites, we performed a Levene’s test. Data were normalized using a Box-Cox transformation (Box and Cox, 1964). These analyses were run using SAS JMP Pro 12.1.0 (Cary, NC).
For the NY site, we evaluated the extent that abiotic factors accounted for variation in the date of root initiation, peak root standing crop, peak root growth rate, or root cessation, including heating degree days, day of year, days before bloom, average daily soil temperature (°C), sum of precipitation over the previous week (mm), and solar radiation (W m⁻²). To examine which factors remained consistent at the date of root initiation among years, we used a generalized linear mixed model using the penalized quasi-likelihood (PQL) approach with a Gaussian distribution and log link function. Plots were repeated across years as a random factor, ‘year’ as the independent variable, and all abiotic factors were separately analyzed as dependent variables. We evaluated whether certain abiotic variables were consistent among years and were thus correlated with the date of root initiation. This method was repeated with the date of peak root standing crop and root cessation. Generalized linear mixed models with blocks repeated across years as a random factor and treatment as the independent variable were used to compare the timing of phenological events between pruning and irrigation treatments. These analyses were run using R 3.2.2 (RCoreTeam, 2015).

**Results**

*Seasonal root production patterns*

For the continental climate site in New York, the largest pulse of root growth occurred between bloom and harvest, regardless of pruning or irrigation treatment, although this comprised only 35% of total yearly root growth (Figs. 2, 4, and 5, Table 1). Very limited root growth occurred in November through April (data not shown). The total length of roots grown in a single year ranged from 10 to 30 m m⁻² in the pruning treatments and 8 to 41 m m⁻² in the irrigation treatments (Figs. 1 through 4). The date of peak root standing crop did not significantly differ among years, and occurred on August 12 (±3.7 days SE, Figs. 1 and 3; F_{4,58}=1.6, P = 0.17).
single local maximum root production rate was recorded during most years, but in 2001 the rate of root production decreased in early July to produce two maxima in August and October, especially in the heavily pruned vines and to a lesser extent in the minimally pruned vines (Fig. 1).

In the New York dataset, the proportion of root growth that occurred during each aboveground phenophase differed significantly among years for all aboveground phenophases (Fig. 5, Table 1; Pre-bloom to bloom: $F_{4,53}=3.8$, $P = 0.008$, bloom to veraison: $F_{4,53}=5.1$, $P = 0.002$, veraison to harvest: $F_{4,50}=3.2$, $P = 0.02$, harvest to end of season: $F_{4,51}=4.4$, $P = 0.004$). The California vines on two rootstocks displayed similar patterns in timing of root production with peaks in production occurring predominately in the early summer months, with the exception of 2005 when the majority of roots were produced in the winter and spring for the less vigorous 101-14 Mgt rootstock (Fig. 6). Overall, the relationship of root production with aboveground phenophases was even more variable among years in the Mediterranean-type climate of California than in New York ($F_{2,41} = 9.3$, $P < 0.001$; Table 2, Fig. 7).

**Interannual variation in root and shoot production**

Averaging eight studies (including the present study), we found that root phenology was about twice as variable across years as aboveground phenology (Table 3, C.V. for roots: 25%, C.V. for shoots: 10%). Additionally, aboveground phenology may be more strongly linked to growing degree days, as growing degree days were less variable for the date of peak shoot growth or bloom than for the date of peak root growth (C.V. for shoots: 27%, C.V. for roots: 45%).

**Root initiation, peak root growth and root standing crop, and root cessation**

Root initiation occurred on a similar degree day across years at the New York site (Fig. 8; $t = -1.84$, $P = 0.08$), which was degree day $121 \pm 9$ (mean $\pm$ SE). Other abiotic variables were
also consistent among years on the date of root initiation, including day of year, soil temperature, solar radiation, and sum of precipitation over the previous week (Fig. 8 and Appendix S2 (see Supplemental Data with the online version of this article); $t = -0.98, P = 0.33; t = -0.89, P = 0.38; t = 1.45, P = 0.16; t = -0.76, P = 0.45$, respectively). These factors may not directly be related to root initiation; however, because variation in the estimates of the means were very high, and this may have masked significant differences among years (see large confidence intervals in Fig. 8). Days from bloom were not related to root initiation, because they varied significantly among years ($t = 2.4, P = 0.03$). The date of root initiation did not differ between pruning or irrigation treatments ($t = -1.23, P = 0.29; t = 1.41, P = 0.23$, respectively).

Peak standing crop occurred at similar days after bloom and sum of precipitation over the previous week each year (Figs. 1, 3, 8, and Appendix S2; $t = 0.36, P = 0.72; t = -0.41, P = 0.68$, respectively). Across years, peak standing crop did not occur at the same day of year, degree day, soil temperature, or solar radiation, ($t = 2.5, P = 0.02; t = 2.6, P = 0.01; t = 2.4, P = 0.02; t = 3.4, P = 0.002$, respectively).

Seasonal peaks of new root production (m m$^{-2}$ day$^{-1}$) varied between treatments and among years in terms of intensity, duration, and date of occurrence (Figs. 2, 4, and 8). The growth rate of the minimally pruned treatment tended to peak before the heavily pruned treatment (day of year 202 ± 4 (mean ± SE) for heavily pruned and day 190 ± 5 for minimally pruned: $t = -2.1, P = 0.055$). This suggests some synchronization with canopy development because minimally pruned canopies develop earlier than heavily pruned canopies (Comas et al., 2005). Unirrigated vines, similarly, tended to peak before the irrigated vines (day of year 201 ± 3 for irrigated and day 188 ± 6 for unirrigated: $t = -2.1, P = 0.05$). The dates of peak new root production in the 101-14 Mgt. rootstock at the California site were day of year 154, 124, and 122 in 2003, 2004, and 2005, respectively, and day 174, 124, and 151 in the 1103P rootstock. Although some variation may be due to data from CA only spanning three years, variation in date of peak root growth was much
greater in California than in New York (standard deviation of 4.3 days in NY, 25 days in the CA 1103P rootstock and 18 days in the 101-14 Mgt. rootstock).

Final cessation of root growth (not including any winter growth in CA) occurred 49 days earlier in CA than in NY on average. The average day of year of cessation in NY was 247 ± 3 (mean ± SE) days, 205 ± 7 days in the CA 1103P rootstock, and 191 ± 19 days in the CA 101-14 Mgt. rootstock. Root cessation in the NY site was linked to degree days, day of year, soil temperatures (which were always 20°C or higher), days after bloom, and the sum of rain over the previous seven days, as these variables were not significantly different on the date of root cessation across years (Figure 8 and Appendix S2; \( t = -0.6, P = 0.54; t = 0.2, P = 0.85; t = 0.56, P = 0.57; t = 0.36, P = 0.72; t = -0.4, P = 0.72 \), respectively). Solar radiation was not similar across years on the date of root cessation (Appendix S2; \( t = 3.0, P = 0.005 \)).

Discussion

Contrary to our expectations, we found limited evidence that root phenology is linked to periods of aboveground phenology, which may be partially due to high variation in the timing of root growth. While there was some evidence of synchronization (both root growth rate and canopy development peaked earlier in minimally pruned vines than in heavily pruned vines), aboveground phenology could not be used as a predictor of root phenology. Although aboveground phenology was fairly consistent from year to year, root phenology varied widely. For example, in a humid-continental climate in New York, the variation in the timing of flowering was 6 days (standard deviation), but variation in the date of root initiation was 26 days.

The lack of long-term datasets makes interannual variability difficult to assess across studies, but these findings are similar to published literature that tracked above- and belowground growth for at least two years (Table 3). Although many excellent studies examine root phenology,
such as Steinaker and Wilson (2008), we only found eight datasets that present both above- and belowground phenology data over more than one year. On average, these studies found that the date of peak root growth was about twice as variable as the date of peak leaf or stem growth or date of bloom (standard deviation of 32 days for root phenology and 15 days for aboveground phenology). While the metrics of above- and belowground growth differed among studies, we believe these studies suggest that belowground phenology is generally more variable than aboveground phenology. It is possible that root sampling methods contribute to the high annual measures of variability in root phenology due to large spatial variability and imperfect sampling of the root system. Because variability among experimental units was smaller than variability among years, we believe this effect was small and that root phenology is more variable than aboveground phenology (see day of peak growth and root cessation in Fig. 8).

Despite interannual variation, most root growth occurred between bloom and veraison, or fruit ripening (Figs. 2, 4, and 5). Just after the beginning of ripening, the sink demand of the crop is extremely strong and may limit root growth by competing for photosynthate supply (Lakso and Eissenstat, 2005). In this study, there were heavy crops of about 15-22 MT/ha, giving estimated harvest indices of about 50-60% of dry matter in the crop. A later study in the same vineyard with varying crop levels found that the percentage of roots produced post-veraison was negatively related to crop level (Eissenstat and Lakso, unpublished data), further supporting this conclusion.

Patterns of grape root growth at the New York site showed that root phenology was not consistently related to periods of above-ground phenology (Table 1, Fig. 5). The amount of root growth that occurred during periods related to aboveground growth was highly variable, making aboveground phenology a poor predictor of root phenology. Above- and belowground phenology may be even less tightly coupled in Mediterranean climates due to differing constraints on shoots and roots. We found greater interannual variation in the proportion of new root growth that
occurred during each aboveground phenophase in the California dataset compared to the seasonally constrained New York dataset (Table 2, Fig. 5). Abramoff and Finzi (2015) found uncoupling of root and aboveground phenology in Mediterranean climates, and they suggest that Mediterranean root growth may be fueled by stored photosynthate, rather than by concurrent photosynthate production. Alternatively, mid-summer root growth in California may be constrained by high temperatures, and winter root growth in New York may be constrained by cold temperatures.

Focusing on root phenology in New York where we had five years of data, we found that the timing of root initiation, peak root standing crop and root growth rate, and root cessation are likely driven by different suites of factors from each other. Differences among phases are suggested initially by disparities in interannual variability in the date of these events, where, for example, the date of root initiation was about six times more variable among years than the date of peak root standing crop (Table 3).

Root initiation may be driven by a combination of factors, including degree days, day of year, and soil temperature, but high variability within and among years limited efforts to predict the timing of root initiation using these variables. Because the confidence intervals within years were smaller than the variation in the date of root initiation among years (Fig. 8), it is unlikely the high interannual variation in root initiation was due to plot-level variation. Although soil temperatures below freezing may constrain root growth, soil temperatures at root initiation varied widely among and within years (Fig. 8; 9.4°C ± 7.0 (mean ± SD)), suggesting that soil temperature is not the sole or primary control. Root initiation may also be controlled by the previous year’s abiotic factors or stored carbohydrates, which were not evaluated in this study (Comas et al., 2005; Radville et al., 2016).

The timing of peak root standing crop may be more constrained by above-ground phenology than root initiation, because peak standing crop in New York occurred at similar days
after bloom across the five years of observation (Figure 4). Peak root growth may be linked to
days after bloom because the plant needs to balance carbon used in root production with carbon
used in reproductive structures as mentioned previously. In addition to carbon constraints, periods
of maximum root production may face pressure to coincide with optimal environmental
conditions. Peak root standing crop did not occur on the same date or cumulative temperature
(degree days) each year, so these cues are likely to be weak predictors for the timing of peak root
flushes.

The timing of root cessation in the fall occurred at a similar day of year, degree days, soil
temperature, days after bloom, and rainfall across all years of the study. Many of these factors,
such as soil temperature and day of year, may be correlated, so it is impossible to disassociate
these variables, but it is possible that cool soils are a cue for roots to cease production for the
year. Previous research on the influence of soil temperature on root production and cessation
provide mixed results. Several studies suggest that root phenology and soil temperature are
correlated and that temperature may be a primary constraint on root growth (Burke and Raynal,
1994; Steinaker and Wilson, 2008; Steinaker et al., 2010), but other studies failed to find strong
correlations with soil temperature (Joslin et al., 2001; Sword et al., 1996; Tierney et al., 2003).
Seasonal new root production ended in late July in California, about 49 days earlier than in New
York, but root production in CA appears to have the potential for pulses of root growth in winter.
The vines in CA were in a dry, unirrigated system, so root growth may cease earlier in
environments where resource availability, such as water, is scarce. Although we did not measure
soil water availability, other studies suggest that root growth may temporarily cease during
drought (Tierney et al., 2003). Also, the wine grapes in CA are less vigorous than the juice grapes
in NY, which also may be factor contributing to the earlier cessation of root growth in the grapes
in CA.
Our observations suggest that the conventional view of a bimodal pattern of root growth during the growing season should be revisited due to high variability in relative root production among years in two distinctly different climates. Textbook examples often claim that there is one flush of root growth in the spring and a second flush in the fall after harvest (e.g. Freeman and Smart, 1976; Van Zyl, 1988; Williams and Matthews, 1990). This assumption has been challenged, and variable numbers of root flushes have been found in different woody crop species (Atkinson and Wilson, 1980; Comas et al., 2010; Contador et al., 2015; Eissenstat et al., 2006; Psarras et al., 2000). A single root flush was observed most often during the warmest portion of the growing season (May-July) for both climates. The only exception was in 2001 at the humid continental site where there were two maxima: one in June and one in July.

These findings have implications for climate change research, as the timing of aboveground plant growth may shift with increased air temperatures (Post et al., 2008). Heat unit accumulation (degree days) is a primary control on aboveground phenology in *Vitis* spp., and such heat unit accumulation is expected to advance as global temperatures increase (IPCC, 2014). If root production is partially driven by heating degree days, root phenology may advance if temperatures warm earlier in spring. This is supported by observations of winter root growth at the Mediterranean site, which may have been possible due to milder winter temperatures than the New York site. Other differences between sites could also account for site-specific differences in root phenology, such as climate, grape species, and plant age. Changes in timing of root production could be detrimental if production no longer coincides with peak periods of water and nutrient availability or could be beneficial if root growth shifts to align with periods of resource availability.

In conclusion, we have demonstrated that aboveground phenological phases cannot be used to predict broad-scale root phenology in grape. This uncoupling may be due to the high interannual variability of root phenology. We also suggest that different belowground
phenological events, such as root initiation, peak root standing crop, and root cessation should be analyzed separately in studies of root growth and predicted separately in models, because they may be driven by different suites of factors. If we analyze these events separately and conduct long-term experiments to account for interannual variability, we may be able to make further progress in understanding controls on root phenology. Detailed knowledge about the timing of root growth and root flushes is especially valuable to informing vineyard management practices, such as improved timing of fertilizer application. Understanding these controls can help predict the impacts of climate change, because climate change may exacerbate the high interannual variability in root phenology, change the relationship among root phenophases, and uncouple peak root production from periods of resource availability.

**Acknowledgements:** The authors thank helpful comments by two anonymous reviewers and the technical support of Tom Adams, Paula Joy, Christine Stockert, Denise Gardner, Jason Benz, as well as many other current and former members of the Eissenstat, Lakso, Smart, and Fredonia labs. Funding was provided by grants from USDA CSREES Viticulture Consortium-East and Viticulture Consortium-West to ANL, DRS and DME, the American Vineyard Foundation and the California Competitive Grants Program for Research in Viticulture and Enology to DRS and NY Wine & Grape Foundation to ANL and DME. We also wish to thank support from the National Science Foundation (IOB-0613832, ARC-1107381) to DME.
Figure 2-1: Seasonal root standing crop patterns in ‘Concord’ grape, *Vitis labruscana* Bailey, in Fredonia, New York, USA, for two pruning treatments (heavy and minimal with irrigation treatments combined) over five years. Proportion of seasonal root standing crop is the length of roots present on a given date divided by the total root standing crop across the entire season. Standard errors were calculated from the length of roots present on a given date for a given block. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total length of roots (m m$^{-2}$) present between April and November.
Figure 2-2: Seasonal root production in ‘Concord’ grape, *Vitis labruscana* Bailey, in Fredonia, New York, USA, for two pruning treatments (heavy and minimal with irrigation treatments combined) over five years. Proportion of seasonal root production is the length of new root extension on a given date divided by the total root extension in the entire season. Standard errors were calculated from the length of roots born on a given date for a given block. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total lengths of root extension (m m^{-2}) between April and November.
Figure 2-3: Seasonal root standing crop patterns in ‘Concord’ grape, *Vitis labruscana* Bailey, in Fredonia, New York, USA, for two irrigation treatments (irrigated and not irrigated with pruning treatments combined) over five years. Proportion of seasonal root standing crop is the length of roots present on a given date divided by the total root standing crop over the season. Standard errors were calculated from the length of roots present on a given date for a given block. X-axis tick marks represent the first of the month. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total standing crop (length) of roots per unit observation surface of the minirhizotron (m m⁻²) between April and November.
Figure 2-4: Seasonal new root production in ‘Concord’ grape, *Vitis labruscana* Bailey, in Fredonia, New York, USA, for two irrigation treatments (irrigated and not irrigated with pruning treatments combined) over five years. Proportion of seasonal root production is the length of root extension on a given date divided by the total length of root extension in the season. Standard errors were calculated from the length of roots born on a given date for a given block. X-axis tick marks represent the first of the month. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total lengths of root extension (m m⁻²) between April and November.
Figure 2-5: The percent of new root growth that occurred during each of four periods related to aboveground phenophases over five years 1998 to 2002. Data come from ‘Concord’ grape, *Vitis labruscana* Bailey, in Fredonia, New York, USA with irrigation and pruning treatments combined. Error bars represent the minimum and maximum values over the five years, boxes represent the upper bound of the first and third quartiles, and middle line represents the median.
Figure 2-6: Seasonal root production patterns in ‘Merlot’ grape, *Vitis vinifera*, on two rootstocks 1103P and 101-14 Mgt. in Oakville, California, USA, over three years 2003-2005 under no irrigation (0% ETc) calculated as the length of root extension in a given block on a given date divided by the total length of root extension in each year. Standard errors were calculated from the mean percentages of roots born. X-axis tick marks represent the first of the month. Lines represent bloom (B), veraison (V), and harvest (H) dates. Numbers in parentheses are the total length of root extension (m m$^{-2}$) in each year.
Figure 2-7: The percent of new root growth that occurred during each of four periods related to aboveground phenophases over three years from 2003-2005. Data come from ‘Merlot’ grape, *Vitis vinifera*, in Oakville, California, USA. Error bars represent maximum and minimum values and squares represent the median.
Figure 2-8: Relationships of root initiation (the date at which root growth first occurred in a given experimental unit), peak root standing crop (date with maximum length of roots), peak root growth rate (maximum length of new roots produced over a two week period), and root cessation (date at which there were at least three following sampling dates with less than 5% of total root growth) with days before or after bloom, day of year, and soil temperature. Points represent the average abiotic conditions that occurred on these key phenological states. Error bars represent 95% confidence intervals. Data are from 1998-2002 in ‘Concord’ grape, Vitis labruscana Bailey, in Fredonia, New York, USA.
Table 2-1: Percent of yearly new root production during periods related to aboveground phenology of *Vitis labruscana* in Fredonia, New York, USA. Data are from 1998-2002 and represent the mean ± standard error (SE), standard deviation (SD), and coefficient of variation (CV). Variation among years in percent root growth during each phenophase, as indicated by SD, was significant ($P < 0.05$).

<table>
<thead>
<tr>
<th>Period</th>
<th>Mean ± SE</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>January to Bloom</td>
<td>28 ± 3.1</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>Bloom to Veraison</td>
<td>35 ± 2.5</td>
<td>5.6</td>
<td>16</td>
</tr>
<tr>
<td>Veraison to Harvest</td>
<td>21 ± 2.9</td>
<td>6.6</td>
<td>32</td>
</tr>
<tr>
<td>Harvest to November</td>
<td>17 ± 4.2</td>
<td>9.4</td>
<td>56</td>
</tr>
</tbody>
</table>

Table 2-2: Percent of yearly new root production during periods related to aboveground phenology of *Vitis vinifera* in Oakville, California, USA. Data are from 2003-2005 and represent the mean ± standard error (SE), standard deviation (SD), and coefficient of variation (CV). Variation among years in percent root growth during each phenophase, as indicated by SD, was significant ($P < 0.05$).

<table>
<thead>
<tr>
<th>Period</th>
<th>Rootstock 1103 P</th>
<th>Rootstock 101-14 Mgt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>SD</td>
</tr>
<tr>
<td>January to Bloom</td>
<td>23 ± 9.5</td>
<td>17</td>
</tr>
<tr>
<td>Bloom to veraison</td>
<td>69 ± 12</td>
<td>21</td>
</tr>
<tr>
<td>Veraison to Harvest</td>
<td>7.4 ± 5.7</td>
<td>10</td>
</tr>
<tr>
<td>Harvest to November</td>
<td>0.9 ± 0.03</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 2-3: Interannual variation in shoot and root phenology across six studies. The variation in growing degree days (GDD) for dates of phenological events are also presented. On average, the timing of root phenology was more variable than aboveground phenology, and the variation in GDD at peak root phenology was greater than that for aboveground phenology. Variation in phenology is measured as variation in the date at which the phenological event occurred. C.V. represents coefficient of variation, and ‘N/A’ indicates that data were not available.

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Vegetation</th>
<th>Measure</th>
<th>Aboveground organs or roots</th>
<th>Variation in phenology (C.V.)</th>
<th>Variation in GDD (C.V.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average of all studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current study</td>
<td>NY, USA</td>
<td>Concord grape</td>
<td>Bloom</td>
<td>Aboveground</td>
<td>3.4</td>
<td>55.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initiation</td>
<td>Roots</td>
<td>32.1</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak</td>
<td>Roots</td>
<td>5.1</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cessation</td>
<td>Roots</td>
<td>5.2</td>
<td>11.1</td>
</tr>
<tr>
<td>Blume-Werry et al. (2015)</td>
<td>Låktatjåkka, Sweden</td>
<td>High alpine tundra, low alpine tundra, and subalpine birch forest</td>
<td>Peak</td>
<td>Aboveground</td>
<td>4.6</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broschat, (1998)</td>
<td>FL, USA</td>
<td>Coconut palm, pygmy palm, queen palm, and royal palm</td>
<td>Peak</td>
<td>Aboveground</td>
<td>15.5</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eissenstat et al. (2006)</td>
<td>British Columbia, Canada</td>
<td>Gala/M9 and 'Golden Delicious'/M9 apple trees</td>
<td>Bloom</td>
<td>Aboveground</td>
<td>6.3</td>
<td>47.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibacache, Rojas, and Jopia (1999)</td>
<td>La Serena, Chile</td>
<td>Cherimoya trees</td>
<td>Peak</td>
<td>Aboveground</td>
<td>37.1</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Location</td>
<td>Species</td>
<td>Peak Aboveground</td>
<td>Peak Roots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------------</td>
<td>------------------------</td>
<td>------------------</td>
<td>------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psarras et al. (2000)</td>
<td>NY, USA</td>
<td>‘Mutsu’/M9 apple trees</td>
<td>9.7</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rytter (2001)</td>
<td>Uppsala, Sweden</td>
<td>Salix viminalis</td>
<td>4.9</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tingey et al. (1996)</td>
<td>Placerville, CA</td>
<td>Pinus ponderosa</td>
<td>1.4</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References


Sword MA, Gravatt DA, Faulkner PL, Chambers JL. 1996. Seasonal branch and fine root growth of juvenile loblolly pine five growing seasons after fertilization. Tree Physiology 16, 899-904.


Chapter 3

**Root phenology in an Arctic shrub-graminoid community: The effects of long-term warming and herbivore exclusion**

Laura Radville$^1$, Eric Post$^2$, David M. Eissenstat$^1$

$^1$Department of Ecosystem Science and Management and the Ecology Graduate Program, The Pennsylvania State University, University Park, PA, USA

$^2$Department of Biology and the Ecology Graduate Program, The Pennsylvania State University, University Park, PA, USA

Published: Climate Change Responses (2016) 3:4

**Abstract**

**Background:** Shifts in phenology have been widely reported in response to global warming and have strong effects on ecosystem processes and greenhouse gas emissions. It is well documented that warming generally advances many phenophases of aboveground plant phenology, but its influence on root phenology is unclear. Most terrestrial biosphere models assume that root and shoot growth occur at the same time and are influenced by warming in the same manner, but recent studies suggest that this may not be the case. Testing this assumption is particularly important in the Arctic where over 70% of plant biomass can be belowground and warming is happening faster than in other ecosystems. Herbivory may mediate the impacts of warming, and carbon removal from grazing may alter carbon available for root growth. In 2013 and 2014 we examined the timing of root growth in Arctic shrub-graminoid communities in a fully factorial
design of plots that were warmed or ambient and excluded or permitted access by large herbivores.

**Results:** Peak root growth occurred two and one half weeks before leaf growth, suggesting that spring root phenology is not controlled by carbon produced during spring photosynthesis. This may uncouple spring root phenology from spring shoot phenology. Consistent with such uncoupling, spring leaf cover was advanced by warming and delayed by herbivory, but neither treatment significantly affected root phenology. Root growth was not driven by soil temperature, and occurred in near-freezing temperatures above the permafrost. Additionally, summer root production appeared to be linked to soil moisture at this relatively dry site, and autumn phenology was not driven by photoperiod as previous studies have suggested.

**Conclusions:** Root phenology was not directly driven by temperature in this system, promoting differential above- and belowground phenological responses to warming and herbivore exclusion. Aboveground phenology, one of the most widely measured aspects of climate change, may not represent whole-plant phenology or indicate the timing of whole-plant carbon fluxes as commonly assumed.

**Keywords:** root phenology, Arctic, climate change, herbivory
Introduction

The timing of plant phenology is important to ecosystem function, species interactions, and patterns of carbon exchange (Badeck et al., 2004; Cleland et al., 2007; Richardson et al., 2013; Wolkovich et al., 2014). Shifts in plant phenology are one of the more sensitive indicators of climate change and have been widely reported in response to climate change. For example, spring phenology has advanced by about 2.5 days per decade in Europe with recent warming (Menzel et al., 2006). While temperature may be the most important environmental factor controlling the timing of aboveground growth (Wielgolaski, 1999), the effects of warming on belowground phenology are not well understood.

These impacts are particularly important in the Arctic, where over 70% of biomass can be belowground and warming is occurring at twice the global rate (McBean et al., 2005; van Wijk et al., 2003). In the Arctic, impacts of climate change may be more pronounced than in other regions (Anisimov et al., 2007; Post et al., 2009). The Arctic is important both as a sink for carbon dioxide and as a source of methane, but the total effect of warming on future carbon cycling in the Arctic is unclear (McGuire et al., 2009). Currently, several studies have documented aboveground advances in phenology in the Arctic (Bjorkman et al., 2015; Hoye et al., 2007; Hoye et al., 2013; Kerby and Post, 2013; Post and Forchhammer, 2008; Zeng et al., 2013), but they do not account for belowground growth.

We currently do not have clear understanding of how factors such as temperature, moisture, and day length impact root phenology, and this limits understanding of how warming will impact whole-plant phenology. Because root phenology and soil temperature are correlated (Burke and Raynal, 1994; Steinaker and Wilson, 2008; Steinaker et al., 2010), increased temperatures should promote higher rates of root production (Wan et al., 2004), reduced root lifespan (Chen and Brassard, 2013), and an extended root growing season (Majdi and Ohrvik,
2004). However, studies that failed to find a correlation between root phenology and soil temperature suggest that endogenous factors, such as photosynthate availability, may be more important to root phenology (Joslin et al., 2001; Sword et al., 1996; Tierney et al., 2003). Soil moisture may also be important, because, according to at least one study, fine root growth increases exponentially with increasing soil water content (Du and Fang, 2014). Although the influence of endogenous and exogenous factors on root phenology is unclear, it is likely that both control root production to some extent (Steinaker and Wilson, 2008; Tierney et al., 2003).

Accurate predictions of whole-plant responses to global warming require a better understanding of the biotic and abiotic factors that control root phenology. Current climate models assume root and shoot growth are synchronous and controlled by the same factors, but this may not be the case (Abramoff and Finzi, 2015; Blume-Werry et al., 2015).

We also have a poor understanding of the relationship between above- and belowground growth and how the relationship is affected by warming. Most climate models include root carbon allocation as a fixed, synchronous fraction of aboveground carbon, but, in many ecosystems, root growth is asynchronous with shoot growth. With decreasing annual temperatures, some studies show a greater asynchrony between root and shoot growth, with root growth occurring much later than shoot growth (Abramoff and Finzi, 2015). For example, in woody plants in the sub-Arctic, root growth peaked about a month later than leaf growth (Sloan et al., 2016). Above- and belowground phenology may be linked, however, as root and leaf turnover are positively correlated in a variety of arctic communities (Sloan et al., 2013). The link between above- and belowground growth is important to understand in regions that experience seasonal herbivory, such as arctic regions exposed to migratory caribou (Rangifer tarandus) populations. Through removal of aboveground biomass, herbivores may reduce carbon available for root growth and may mediate root responses to climate change. The influence of herbivory on root growth varies across ecosystems, and herbivory may mitigate the effects of warming on plant
community composition (Post and Pedersen, 2008). By examining the influence of herbivory, we can examine how natural removal of aboveground biomass, or of herbivore mediation of interspecific interactions among plants, affects the timing of root growth.

In 2013 and 2014 we examined the influence of warming and herbivory on the timing of root and shoot growth in an arctic system and evaluated environmental controls on these, including soil temperature, soil moisture, and photoperiod. We examined the influence of soil temperature on root phenology at different soil depths throughout the season. We also characterized the relationship between above- and belowground phenology, particularly in response to both warming by open-top chambers and herbivory by large mammals. Minirhizotrons were used to non-destructively evaluate the timing of root growth. This study took place near Kangerlussuaq, Greenland, where mixed-vegetation plots have experienced a warmed/unwarmed treatment (with open-top chambers) and herbivore absence/presence (with fences) for 13 years (Post, 2013) We hypothesized that warming would advance both above- and belowground growth, and herbivory would reduce aboveground growth and delay belowground growth. We also hypothesized that above- and belowground phenology would be offset, with root growth occurring after shoot growth, as observed in previous studies (Abramoff and Finzi, 2015; Steinaker et al., 2010).

Methods

Experimental site and design

This study took place in low shrub tundra near Kangerlussuaq, Greenland (67.11°N, 50.37°W). This site is on dry acidic tundra on noncarbonated bedrock in Arctic shrub-tundra (Elvebakk, 1999). The vegetation community is characterized by patches of deciduous shrubs, primarily Salix glauca and Betula nana, and graminoid species, such as Poa pratensis and Carex
This is a permafrost ecosystem, and the average active layer depth at a nearby site was 63 cm between May and August in 2014 (Cahoon et al., 2016), and there is a mossy organic layer in all plots. The mean annual air temperature was -4.1 C in 2013 and -4.5 C in 2014.

In 2013 and 2014, we measured root phenology and aboveground phenology in 24 long-term study plots in a fully factorial design of warming by herbivory. Warming has been achieved with open-top chambers seasonally since 2003 and large herbivores [muskoxen (Ovibus moschatus) and caribou (Rangifer tarandus)] have been excluded with fences since 2002 (N = 6 per warming/herbivore exclusion treatment). The 1.5-m-wide open-top chambers were constructed following ITEX protocols (Henry and Molau, 1997) and were placed on the plots from May-July each year. Minirhizotrons, clear, hollow tubes used to follow seasonal root growth, were installed in two of the three 800 m² experimental herbivore exclosures, so these exclosures were utilized in this study. The study site and experimental design have been described in detail in previous publications (Post and Pedersen, 2008)

Root phenology

One minirhizotron tube was installed in each plot in July 2005. Minirhizotron tubes were clear acrylic tubes buried at a 30-degree angle to the vertical and anchored into the soil with steel rods. Tubes were insulated with foam tubing, and each minirhizotron tube was sealed with a plumbing plug. The aboveground portion of the tube was wrapped with electrical tape, painted white, and covered with a white, aluminum cover to exclude light and prevent solar radiative heating. A minirhizotron camera (Bartz Technology Corporation, CA USA) was lowered into the tube, and images of the roots were taken at 1.3 cm depth intervals along the tube. From May to August 2013 and May to September 2014 we photographed tubes weekly in the shoulder seasons, when rapid changes in growth occurred, and less frequently in mid-season, when change was slow. Ice obscured the view of roots in several images, so these were excluded. Root production
and root standing crop were tracked through the season by tracing images of roots with Rootfly software (Clemson University, Clemson SC). This program determines the length of roots growing against tubes on each date. Root length was divided by area of the tube visible in the image to get standing crop (cm roots·cm⁻² viewing surface). To calculate root production, only the length of new root initiation and elongation occurring between two consecutive dates was divided by viewing surface area, which was then divided by the number of days since the previous measurement (cm roots·cm⁻² viewing surface·day⁻¹). In order to examine the influence of depth on the timing of root growth, roots were further separated into categories according to the soil depth at which roots were produced (1-10 cm, 11-20 cm, 21-30 cm, and 31-40 cm).

Abiotic conditions

We measured mid-day (between 11 am and 1 pm) soil moisture and temperature in all plots on each date that root images were obtained. Soil temperature was measured with a thermocouple placed at 5 cm below the organic layer in 2013, and 5- and 10 cm below it in 2014. The organic layer thickness (approximately 5 cm) and mineral soil physical and chemical properties were similar among plots. In both years, soil moisture was measured with a TDR (time domain reflectometry) waveguide placed from 0-10 cm below the organic layer. Continuous meteorological measurements, including air temperature (°C, 2 m above soil surface), precipitation (mm), soil temperature (°C, 0.1, 0.2, and 0.3 m below soil organic layer), and volumetric soil water content (0.1 m below soil organic layer) were collected hourly at a weather station located within the study site using a CR-1000 datalogger, in place since 2008. Day length was calculated for each day using sunrise and sunset times for Kangerlussuaq, Greenland. Soil temperature was averaged hourly at 10, 20, and 30 cm below the organic layer using copper-constantan thermocouples. In order to estimate maximum daily soil temperature at each of these depths in the study plots, we used estimation techniques from Campbell and Norman (1998). Data
from the weather station were used to determine the monthly damping depth for these soils
(Damping depth = (depth 1 – depth 2) / [ln(amplitude of temperature at depth 2) – ln(amplitude of
temperature at depth 1)], where depth 1 was 10 cm and depth 2 was 20 cm). The amplitude of
temperature variation in each plot was estimated as the difference between the measured soil
temperature and the average seasonal air temperature. The relationship between the daily
observed maximum temperature and daily soil temperature at 11 am was determined from the
weather station data for all three depths. Once the temperature at each depth at 11 am was
determined, these regression relationships were used to estimate the maximum daily soil
temperature at each depth in each plot.

Aboveground phenology

We used NDVI (normalized difference vegetation index) to estimate the relative area in
each plot covered by photosynthetically active vegetation. In this study, NDVI = (R800-
R660)/(R800+R660), where R800 is the reflectance at 800nm, and represents a near-infrared
wavelength, while R660 is reflectance at 660nm and represents a photosynthetically active
wavelength (as in Boelman et al., 2003). NDVI was determined from measurements of incident
and reflected light obtained with a Unispec-DC (PP Systems, Haverhill, MA USA). The
spectrometer records radiance from 300-1100nm. The Unispec-DC was centered 2m above each
plot and had a measurement footprint of 0.39m². Measurements were obtained on each day root
images were taken, and three measurements were averaged over each plot. At the beginning of
each day, the Unispec-DC was calibrated with a white standard to account for daily light
conditions.

Statistical analyses
To examine the timing of root production and to remove noise associated with high variation among plots in absolute root production, data were normalized to the maximum value for that plot during that year. For example, the proportion of maximum root production on a given date is calculated by: root production on that date divided by maximum cumulative root production occurring in that plot during that year. The proportion of maximum NDVI and the proportion of maximum root standing crop for each plot were obtained in the same way. The date of peak root production for each plot was measured as the date of maximum root production over the previous sampling date in each year. The date of peak NDVI was the date at which the maximum vegetation cover occurred in each plot and in each year. In plots where the vegetation cover reached a maximum amount and remained at this value, the date of peak NDVI was recorded as the first date that reached this maximum value.

To examine seasonal differences between treatments in volumetric soil water content, soil temperature, proportion of maximum root production, proportion of maximum root standing crop, and NDVI, each of these variables was analyzed with a mixed model using time, treatment, and time*treatment as fixed effects. Plot nested within date was included as a random effect in order to account for non-independence of plots measured repeatedly through time. Non-normally distributed variables were transformed with a Box-Cox transformation (Box and Cox, 1964). To examine correlations between root growth and abiotic factors, we used Pearson product-moment correlations to compare the daily proportion of maximum root production, soil temperature, soil moisture, Julian date, day length, and NDVI. All analyses were performed in SAS JMP Pro 10.0.2 (2012, Cary, NC)

The percent graminoid cover in each plot was used as a covariate in all analyses of NDVI because NDVI was significantly different among vegetation types. Forty-two percent of plots had greater than 50% cover of *Betula nana*, and these plots had an average NDVI of 0.64±0.02 (SE). Twenty-five percent of plots had greater than 50% cover of *Salix glauca*, and these had an NDVI
of 0.61±0.04 (mean±SE). Sixteen percent of plots were dominated by graminoids, and these had an NDVI of 0.49±0.04 (mean±SE). The percent of graminoid cover in each plot was also used as a covariate in analysis of the herbivore exclosure treatment because vegetation type was significantly different inside and outside the exclosures (Post and Pedersen 2008). Inside the herbivore exclosures, 2.5±1% (mean±SE) of each plot was composed of graminoids, but 40±6% (mean±SE) of each plot outside the exclosure was graminoid-covered. Graminoid cover was not a significant factor for the warming treatment. Average graminoid cover in warmed plots was 24±5.6% (SE), and average graminoid cover in ambient plots was 19±4.6% (SE).

Results

The timing of root growth was asynchronous with that of shoots (Figure 1). Contrary to some studies, such as Blume-Werry et al. (2015) in the Arctic, but in accordance with other studies, such as McCormack et al. in a temperate ecosystem (McCormack et al., 2015), root growth preceded shoot growth. Peak root standing crop occurred 18 days earlier than peak aboveground cover. Both root production and root standing crop peaked on July 3 (Julian date 184 ± 5; mean ± SE), and leaf cover peaked on July 21 (Julian date 202 ± 3). The dates of peak root standing crop, peak root production, and peak vegetation cover did not differ by warming or exclosure treatments (Figure 2, Table 2). There was high variation around the peak root production that may have masked treatment differences, as ambient plots peaked on day 180 ± 28 (SD) and warmed plots peaked on day 188 ± 35 (SD). The average total root production was 1.9±0.3 cm cm$^{-2}$ (±SE) in 2013 and 2.1±0.2 cm cm$^{-2}$ (±SE) in 2014. The maximum standing crop was 5.4 cm cm$^{-2}$ in 2013 and 4.4 cm cm$^{-2}$ in 2014. Neither warming nor herbivore exclosure significantly changed the timing of maximum root standing crop across the whole season (Figure 2; Table 2). Variation was high among plots and this may have masked treatment differences. All
treatments followed the general trend in Figure 1, with root production preceding the majority of shoot production. In support of the asynchrony between leaf and root phenology, daily root production was negatively correlated with leaf cover (Table 1).

Both treatments altered the timing of seasonal leaf production, measured as a proportion of the maximum yearly leaf cover (Figure 2, Table 2), although the direction of change differed between years. The amounts of absolute leaf cover (the NDVI values without converting to proportions) differed between treatments in consistent ways. Warmed plots had more vegetation cover early in the season (June 17 to July 1 in 2013 and May 26 to June 15 in 2014; 2013: $F_{1,55} = 39.4, p < 0.001$; 2014: $F_{1,88} = 11.3, p = 0.001$). Herbivory reduced early-season leaf cover in both years (2013: $F_{1,55} = 3.3, p = 0.08$; 2014: $F_{1,70} = 4.5, p = 0.04$).

Contrary to a previous study of autumn phenology in *Dupontia fischeri* and *Eriophorum angustifolium*, conducted in the Arctic using phytotrons in the field and in the laboratory (Shaver and Billings, 1977), root growth did not appear to be constrained by day length. Root growth per day ($\text{cm cm}^{-2} \text{day}^{-1}$) over the last three dates of the season (July 29 to August 12 in 2013 and August 5 to September 5 in 2014) did not decrease as day length decreased (Table 1). Also, root production per day increased at the end of the season in both years (Figure 3), and root production per hour daylight ($\text{cm cm}^{-2} \text{hr daylight}^{-1}$) did not change at the end of 2013 and increased at the end of 2014 (2013: $F_{2,68} = 1.1, p = 0.34$; 2014: $F_{2,69} = 12.4, p < 0.001$). If day length constrained root production, we would expect root production per day to decrease over this interval. We did not find evidence for a lagged effect of day length on root growth, because daily root production was uncorrelated with day length at the previous measurement (Spearman’s $\rho = -0.064, p = 0.45$).

Root phenology did not appear to track temperature in this cold environment. Root production was, instead, positively correlated with soil water content (Table 1). Although soil temperature and soil water content were negatively correlated (Spearman’s $\rho = -0.29, p < 0.001$), soil temperature alone was not correlated with root production (Table 1). The modeled maximum
soil temperature was significantly colder deeper in the soil and deeper soil warmed more slowly throughout the season (Figure 3, Table 2, \( p < 0.001 \)), but root phenology was not significantly different among soil depths (Table 2, \( p = 0.16 \)).

Neither warming nor the herbivore exclosure treatment significantly affected soil water content or soil temperature (Table 2). Soil moisture was highest at the first sampling date of the season, immediately after snowmelt, and reached a low in late July. In 2014, when measurements were taken into September, we observed a slight increase in soil moisture in late August and early September (Figure 4A). Soil temperature at 10cm below the organic layer was close to 0°C in late May and reached a maximum of about 8-10°C in late July in both years (Figure 4B). The period May - September, 2013, was cooler and wetter than the same period in 2014: on average air temperature in 2013 was 4.8°C ± 0.1 (±SE) with 104.1mm ± 0.003 total precipitation and air temperature in 2014 was 7.3°C ± 0.1 with 74.2mm ± 0.003 total precipitation.

**Discussion**

In this highly seasonal environment, soil temperature was not correlated with root phenology. If root growth were solely, or even primarily, limited by temperature, we would expect shallow roots to commence growth before deep roots because soils warm more slowly in deep soil layers. We did not see this trend, and deeper roots grew very early in the season even though estimated maximum temperatures were only slightly above 0°C (Figure 3). Other studies in the Arctic have also documented root growth at soil temperatures of 1-2°C (Billings et al., 1977; Kummerow and Ellis, 1984; Shaver and Billings, 1977). Shaver and Billings (Shaver and Billings, 1977) suggested that temperatures above 0°C may not be limiting to cold-adapted species, and hypothesized that other factors, such as soil nutrients, may be more limiting. Sullivan et al. (2015) suggest that soil nutrient availability may be more important than growing season
temperatures in limiting growth of arctic plants (Sullivan et al., 2015). We did not directly measure soil nutrients, but nutrient availability and mineralization in the Arctic are directly related to water availability and movement (Oechel, 1989). Although soil temperature may limit root phenology in some ecosystems (Steinaker et al., 2010), our results suggest that other factors, such as soil moisture, were likely of primary importance in controlling root phenology at this site. Temperature may be a secondary or weak control on root phenology in species that are adapted to cope with extreme temperatures.

Previous experimental evidence indicated that late season Arctic root growth is controlled by photoperiod in some species (Shaver and Billings, 1977), but we found that late season root growth did not decrease with decreasing day lengths (Table 1). In wet tussock tundra, Shaver and Billings (1977) suggested that in species with annual roots, photoperiod may cue remobilization of carbohydrates aboveground before soil freezes. In dry tundra species of this study, where many roots are perennial, it may be more important to slightly increase root production in late fall to prepare roots for the spring pulse of water and nutrients. Factors that control root production in the Arctic, such as photoperiod, may differ between ecosystems and species and may depend on limiting conditions at each site.

Leaf and root phenology were asynchronous, and peak root growth occurred 18 days before peak leaf cover (Figure 1). These results appear to be in opposition to patterns documented in a meta-analysis in which peak root growth followed that of shoot growth by 48 days in the boreal zone (Abramoff and Finzi, 2015). Some of this discrepancy could be due to the use of alternate methods of papers comprising the basis of that meta-analysis (such as sequential coring, as opposed to nondestructive minirhizotrons). Alternatively, root phenology in different ecosystems may be adapted to occur when conditions are most favorable for nutrient and water acquisition. We reported a positive correlation between root growth and soil moisture, suggesting that soil water content was a strong constraint on root production. In this dry, nutrient-limited
system, soil moisture is highest in early spring, presumably coincident with the timing of snowmelt. It may be most advantageous to proliferate roots when the soil is moist and nutrients are mobile, particularly in arctic ecosystems where nitrogen availability is highly seasonal (Jonasson and Shaver, 1999). In other ecosystems with seasonal constraints on water availability, root growth initiates quickly during periods of high rainfall (Salguero-Gomez and Casper, 2011) and slows or ceases entirely during dry periods (Kuhns et al., 1985; Teskey and Hinckley, 1981). Although we did not directly examine seasonal nutrient availability, it is likely linked to water availability. These results suggest that root phenology may differ widely among ecosystems as roots proliferate during the most favorable periods of the year. These differences may be most apparent in temperature-limited ecosystems, such as the Arctic, where the short growing season requires root phenology to occur during the brief window of favorable conditions.

The asynchrony between root and shoot phenology suggests that above- and below-ground organs are either controlled by different biotic and abiotic factors, or that they compete for carbon-use. A carbon trade-off between roots and shoots has been found in other systems, in which carbon is allocated to leaf and root growth at different times of the year due to constraints on carbon availability (e.g. Reich et al., 1980). In support of a carbon trade-off between root and shoot growth, the two were negatively correlated throughout the season (Table 1). This correlation could not be uncoupled from other factors that also varied throughout the season, however, and may be confounded with periods of high soil moisture. Because roots and shoots differ in the timing of carbon use, they are likely influenced differently by the environment. Warmer spring temperatures, for example, may not influence root phenology if spring root phenology is not driven by temperature or leaf carbon production.

Because root production occurs before leaf photosynthate production, root growth was probably fueled by stored carbohydrates rather than by current photosynthate production, as has been noted in other species (Sloan and Jacobs, 2008). In accordance with this assumption,
warming and herbivore exclusion altered the timing of spring leaf cover but not the timing of root standing crop or production (Figure 2, Table 2). Although both treatments altered aboveground phenology, belowground phenology may have been timed to coincide with early-season water availability and was not driven by photosynthate availability. The second flush of root growth in late fall may be a means of acquiring nutrients that become available in newly fallen leaf litter as well as an adaptation to prepare roots for the pulse of nutrients in early spring. Although these plants seem capable of root growth at least at near-freezing temperatures, they may be mechanically unable to grow in completely frozen soil.

These results may be ecosystem-specific, because other studies in the Arctic found that experimental warming increased root production (Sullivan et al., 2008) and advanced root growth (Sullivan and Welker, 2005). This study was conducted in a much drier environment than prior studies, so our results suggest that water availability and potentially nutrient availability may be stronger drivers of root growth in dry Arctic tundra. Another explanation for these results is that high spatial and interannual variability in root growth masked the effect of treatment on root phenology in this study. For example, the average date of peak root growth differed by 8.6 days between warming treatments, which could be biologically significant, but there were large standard deviations around each mean and the difference was not statistically significant ($p = 0.36$). These responses were measured across the entire plant community, but individual species may have responded differentially to herbivore exclusion and warming. Despite these caveats, these findings provide evidence that current climate model treatment of roots as a fixed, synchronous fraction of aboveground growth are not accurate (Abramoff and Finzi, 2015). Roots may be under different constraints than canopy tissues, and use of carbon stores for spring root growth may mediate the impacts of climate change on root phenology.

Belowground biomass can be up to five times greater than aboveground biomass in the Arctic (Mokany et al., 2006), and the uncoupling of root phenology from leaf phenology makes it
very difficult to estimate whole-plant phenology without intensive root monitoring. Controls on root phenology may differ by ecosystem, and root phenology may not be driven by temperature or spring carbon production in some species. This would uncouple root and shoot phenology further, as production of aboveground tissues is strongly constrained by air temperature.

**Conclusions**

Root phenology was not directly driven by temperature or day length in this ecosystem, and above- and belowground phenology did not respond in the same way to warming and herbivore exclusion. These results suggest that aboveground phenology, one of the most widely measured aspects of climate change, may not represent whole-plant phenology and may be a less accurate predictor of the timing of whole-plant carbon fluxes than commonly assumed.

**Acknowledgements:** This work would not have been possible without the help of Tom Adams, Thomas Bentley, Sean Cahoon, Elizabeth Elmstrom, Kate Lamp, Anthony Siudela, Chénira Smith, and Tyler Tran.
Figure 3-1: The proportion of maximum seasonal root (standing crop) and shoot (NDVI) growth for mixed-vegetation plots in 2013 and 2014. Black lines with circular points indicate the mean proportion of maximum root growth, and gray lines with square points represent the mean proportion of maximum shoot growth. All treatments are combined (N=24). Error bars indicate ±1 SE. The minimum and maximum root standing crops were 0.1 and 5.4 cm cm\(^{-2}\) in 2013 and 0.09 and 4.4 cm cm\(^{-2}\) in 2014. The minimum and maximum NDVIs were 0.07 and 0.88 in 2013 and 0.16 and 0.84 in 2014.
Figure 3-2: The proportion of maximum seasonal root (standing crop) and shoot (NDVI; leaf cover) growth for mixed-vegetation plots in 2013 and 2014, separated by warming and exclosure treatments. 

**A.** Proportion maximum shoot and root growth in 2013 and 2014, separated by exclosure treatment with warming treatments combined. Black dashed lines with closed circles indicate root standing crop with no herbivory, gray dashed lines with open circles are root standing crop with herbivory, gray solid lines with open squares are NDVI with herbivory, and black solid lines with closed squares are NDVI without herbivory. The timing of leaf cover was significantly different between plots with and without herbivory, but the timing of root standing crop was not.

**B.** Proportion maximum shoot and root growth in 2013 and 2014, separated by warming treatment with exclosure treatments combined. Black dashed lines with closed circles indicate ambient root standing crop, gray dashed lines with open circles are warmed root standing crop, gray solid lines with open squares are warmed NDVI, and black solid lines with closed squares are ambient NDVI. The timing of leaf cover was significantly different between warmed and unwarmed plots, but the timing of root standing crop was not. Sample size (N) was 12 for each of the two treatments (Error bars indicate ±1 SE).
Figure 3-3: Grey area on left y-axis represents mean daily new root production (cm cm$^{-2}$ day$^{-1}$) in mixed-vegetation plots in 2014, separated by soil depth. The date of peak root production was June 2 in 2014 and the average total root production from May 17 to September 5 2014 was 2.1±0.2 cm cm$^{-2}$ (±SE). The black line on the right y-axis is the estimated soil temperature for each depth. Error bars indicate ±1 SE. All treatments are combined (N=24). Top row represent roots 1 to 10 cm below soil organic layer, second row represents roots 11-20 cm below soil organic layer, and bottom row represents roots 21-30 cm below soil organic layer. Figure is adapted from Radville et al. (2016).
Figure 3-4: A. The average daily volumetric soil water content from 0-10 cm in mixed-vegetation plots in 2013 and 2014. B. The average daily soil temperature at 5 cm in mixed-vegetation plots in 2013 and 2014. Error bars indicate ±1 SE and N=24.
Table 3-1: Correlations (Spearman’s ρ) with daily root production (cm cm⁻² day⁻¹). End of season represents July 29 to August 12 in 2013 and August 5 to September 5 in 2014.

<table>
<thead>
<tr>
<th>Duration</th>
<th>NDVI</th>
<th>Soil water content</th>
<th>Soil temperature</th>
<th>Day length</th>
<th>Julian day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole season</td>
<td>0.37 ***</td>
<td>0.26 **</td>
<td>-0.067 (ns)</td>
<td>0.30 ***</td>
<td>0.11 *</td>
</tr>
<tr>
<td>End of season</td>
<td>0.13 (ns)</td>
<td>0.16 (ns)</td>
<td>0.0079 (ns)</td>
<td>0.062 (ns)</td>
<td>0.062 (ns)</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01, *** p < 0.001, ns = not significant
Table 3-2: Peak date of root production is the date on which new root growth over the previous week was highest. Peak date of root standing crop is date with largest total length of roots. Peak date of vegetation cover is date with largest NDVI. The percent of maximum root production is a metric of seasonality, calculated as the new root growth on each date divided by the maximum root production in that plot and year. Percent of maximum standing crop and NDVI are calculated in the same way. “Warming” denotes warming/ambient treatments, “Exclosure” denotes herbivores present/absent, and “Depth” is a comparison of 0-10cm, 10-20cm, and 20-30cm.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Treatment</th>
<th>DF num</th>
<th>DF den</th>
<th>F ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak date of root production</td>
<td>Warming*date</td>
<td>1</td>
<td>44</td>
<td>0.85</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Exclosure*date</td>
<td>1</td>
<td>44</td>
<td>0.26</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Warming*Exclosure</td>
<td>1</td>
<td>44</td>
<td>0.082</td>
<td>0.78</td>
</tr>
<tr>
<td>Peak date of root standing crop</td>
<td>Warming*date</td>
<td>1</td>
<td>41</td>
<td>0.82</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Exclosure*date</td>
<td>1</td>
<td>41</td>
<td>0.25</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Warming*exclosure</td>
<td>1</td>
<td>41</td>
<td>0.073</td>
<td>0.79</td>
</tr>
<tr>
<td>Peak date of vegetation cover (NDVI)</td>
<td>Warming*date</td>
<td>1</td>
<td>44</td>
<td>0.013</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Exclosure*date</td>
<td>1</td>
<td>44</td>
<td>0.82</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Warming*exclosure</td>
<td>1</td>
<td>44</td>
<td>0.72</td>
<td>0.4</td>
</tr>
<tr>
<td>Percent of maximum root production</td>
<td>Depth*date</td>
<td>36</td>
<td>653</td>
<td>1.2</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Warming*date</td>
<td>18</td>
<td>384</td>
<td>1.2</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Exclosure*date</td>
<td>18</td>
<td>384</td>
<td>0.46</td>
<td>0.95</td>
</tr>
<tr>
<td>Percent of maximum root standing crop</td>
<td>Warming*date</td>
<td>19</td>
<td>410</td>
<td>0.74</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Exclosure*date</td>
<td>19</td>
<td>410</td>
<td>1.5</td>
<td>0.066</td>
</tr>
<tr>
<td>Percent of maximum vegetation cover (NDVI)</td>
<td>Warming*date</td>
<td>16</td>
<td>341</td>
<td>3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Exclosure*date</td>
<td>16</td>
<td>341</td>
<td>2.1</td>
<td>0.0066</td>
</tr>
<tr>
<td>Soil temperature (C)</td>
<td>Depth</td>
<td>2</td>
<td>601</td>
<td>1108</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td><strong>Depth</strong></td>
<td>18</td>
<td>601</td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Warming</td>
<td>1</td>
<td>22</td>
<td>0.0008</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Warming*date</td>
<td>17</td>
<td>383</td>
<td>1.2</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Exclosure</td>
<td>1</td>
<td>22</td>
<td>0.55</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Exclosure*date</td>
<td>17</td>
<td>383</td>
<td>0.65</td>
<td>0.85</td>
</tr>
<tr>
<td>Soil moisture (volumetric proportion)</td>
<td>Warming</td>
<td>1</td>
<td>22</td>
<td>0.11</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Warming*date</td>
<td>16</td>
<td>375</td>
<td>0.71</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Exclosure</td>
<td>1</td>
<td>22</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Exclosure*date</td>
<td>16</td>
<td>375</td>
<td>1.04</td>
<td>0.41</td>
</tr>
</tbody>
</table>
References


Post E, Forchhammer MC. 2008. Climate change reduces reproductive success of an Arctic herbivore through trophic mismatch. Philosophical Transactions of the Royal Society B-Biological Sciences 363, 2369-2375.


Sword MA, Gravatt DA, Faulkner PL, Chambers JL. 1996. Seasonal branch and fine root growth of juvenile loblolly pine five growing seasons after fertilization. Tree Physiology 16, 899-904.


Chapter 4

On the sensitivity of root and leaf phenology to warming in the Arctic

Laura Radville¹, Eric Post², David M. Eissenstat¹

¹Department of Ecosystem Science and Management and the Ecology Graduate Program, The Pennsylvania State University, University Park, PA, 16802, USA

²Department of Wildlife, Fish, & Conservation Biology, University of California, Davis, Davis, CA, 95616, USA

Submitted to Arctic, Antarctic, and Alpine Research

Abstract

Temperature is commonly assumed to act as the primary constraint on the timing of plant growth, and strong advances in plant phenology have been seen with recent atmospheric warming. The influence of temperature on the timing of root growth, however, is less clear, and controls on root phenology are not well understood. The influence of temperature on above- and belowground phenology is particularly important in the Arctic, where most plant biomass is belowground and warming is occurring at a higher rate than in other ecosystems. We examined the influence of experimental warming on graminoid and shrub communities in the Arctic in southwestern Greenland. We found that warming since 2012 did not advance the timing of aboveground phenology over two years or belowground phenology over three years. We suggest that warming may not be the primary constraint on phenology in all ecosystems, and the direct influence of warming in these environments may be weaker than expected.
**Introduction**

Shifts in the timing of plant growth, or phenology, are one of the strongest indicators of global warming, and they can have strong effects on ecosystem processes and greenhouse gas emissions (Post and Forchhammer, 2008; Richardson et al., 2013; Ernakovich et al., 2014; IPCC, 2014). Temperature is often cited as a primary factor controlling the timing of aboveground growth (Wielgolaski, 1999) and is credited with an advanced spring phenology of 2.5 days per decade in Europe (Menzel et al., 2006). The Arctic is warming at twice the global rate, and the impacts of climate change maybe stronger there than in other ecosystems (McBean et al., 2005; Anisimov et al., 2007; Post et al., 2009). Current models predict that arctic warming will continue to be strong, especially given positive feedbacks between sea-ice melt and local air temperature (Flanner et al., 2011; Vihma, 2014). This warming influences the carbon budget, and earlier plant growth may increase yearly carbon uptake in the Arctic and elsewhere (Cahoon et al., 2016b). It is commonly assumed that warmer arctic temperatures will continue to cause earlier green-up and an increase in vegetation types that grow earlier in the year, such as shrubs. The effect of warming on individual species may be non-linear, however, where initial changes in temperature elicit much stronger effects than later changes (Fu et al., 2015).

Current estimates of shifts in plant phenology in response to climate change are, however, based solely on aboveground phenology and do not account for potential changes in root phenology. Although most terrestrial biosphere models assume roots will respond to warming in the same way as shoots, experimental evidence suggests this may not be the case (Abramoff and Finzi, 2015). Because roots can account for up 70% of total plant biomass in regions of the Arctic (van Wijk et al., 2003), it is essential to account for both above- and belowground phenological responses to warming. If above- and belowground phenology respond
differently to warming, estimates of future carbon exchange driven by presumed phenological dynamics may be inaccurate.

In this study, we experimentally examined the influence of warming on both above- and belowground growth in southwestern Greenland. We recorded phenology of both graminoid (*Poa* spp.; *Carex* spp.) and shrub species (*Betula nana*) over three growing seasons. We expected that warmer temperatures would cause both leaves and roots to grow earlier in the year and that shrubs would be more responsive to warming than grasses.

**Methods**

*Study site and design*

This experiment was conducted near Kangerlussuaq in southwestern Greenland (67.11°N, 50.30°W). The study was set up on dry acidic tundra on noncarbonated bedrock in Arctic shrub-tundra (Elvebakk, 1999). In this permafrost ecosystem the average active layer depth was 63 cm between May and August in 2014 (Cahoon et al., 2016a). The mean annual air temperature was -4.4°C in 2014 and -7.7°C in 2015. In 2016, from January 1 to June 26 the average temperature was -4.13°C. Vegetation types occur in easily distinguished patches at this site, primarily of *Betula nana*, *Salix glauca*, and mixed graminoid species, including *Poa pratensis* and *Carex* spp.

In 2012 we selected 48 plots, including 16 plots that were 100% *Betula nana*, 16 plots that were 100% graminoid species, and 16 plots that were 50% of each vegetation type, which we refer to as “mixed” plots. Half of these plots were on a slightly south-facing slope while the others were on a west-facing slope. The two slope types were approximately 100m apart. Of each vegetation type (shrub, graminoid, and mixed), half were warmed (n=8 per vegetation by warming treatment). Warming was achieved with open-top chambers (OTCs) from May to
August from 2012 to 2016, and through September in 2014. The 1.5-m-diameter passive warming chambers were constructed according to ITEX protocols (Henry and Molau, 1997). Matching ambient, unwarmed plots were also 1.5m in diameter.

Root phenology

Two minirhizotron tubes were installed in each plot in 2012. Minirhizotron tubes were constructed of clear acrylic cylinders buried at a 30-degree angle to the vertical and anchored to the ground with steel rods. To seal the tubes from weather and light and prevent solar radiative heating, all tubes were sealed at the surface end with a plumbing plug, wrapped with electrical tape, painted white, and shielded with a white, aluminum cover. The inside of the tube was filled with removable tubular insulation during non-measurement periods to prevent temperature changes inside the tube.

To monitor root growth, a minirhizotron camera (Bartz Technology Corporation, CA USA) was lowered into the tube and root images were captured at 1.3 cm depth intervals along the tube. Each tube was photographed once per week from 2014-2016, with all tubes being imaged over the course of each week. Because we could not photograph all minirhizotron tubes in one day each week, values of root growth of all tubes were grouped by week in order to statistically analyze plot replicates and to visually represent data. Ice obscured the view of roots in some images, so these were excluded from all analyses. Seasonal root production and root standing crop were quantified by tracing images of roots with Rootfly software (Clemson University, Clemson SC). We determined the length of roots visible on tubes on each date to obtain standing crop (cm roots • cm\(^{-2}\) viewing surface). To calculate root production, the length of new root initiation and elongation occurring between two consecutive dates (cm roots • cm\(^{-2}\) viewing surface), was divided by the number of days since the previous measurement (cm roots • cm\(^{-2}\) viewing surface • day\(^{-1}\)). New roots were reliably identified by their bright white appearance.
Root standing crop was recorded as the length of all roots present in each tube on each date (cm roots \( \cdot \) cm\(^{-2}\) viewing surface).

**Abiotic conditions**

In order to measure soil temperature at different depths, thermocouples were buried at 10, 20, 30, and 40 cm from the bottom of the organic layer in all plots. To measure soil moisture at these depths, time domain reflectometry (TDR) wave guides were buried from 10-20, 20-30, 30-40 cm where 0 cm is top of the mineral soil (bottom of the organic layer) in all plots. To measure temperature and moisture at 0-10 cm where 0 cm is the top of the mineral soil layer, a thermocouple and a TDR probe were manually inserted into the soil. Measurements were taken at mid-day on each date that root images were obtained from 2014-2016. The organic layer depth (approximately 5 cm) and mineral soil physical and chemical properties were similar among plots. In addition, automated measurements of air temperature, soil temperature, soil moisture, and humidity were obtained in 12 plots (n=2 per warming by vegetation type). In these plots, Campbell CR-1000 dataloggers scanned sensors every 30 seconds and stored hourly averages of air temperature (\(^\circ\)C, 10 cm above soil surface), soil temperature (\(^\circ\)C, 10 cm below soil organic layer), and volumetric soil water content (10 cm below soil organic layer) beginning in June 2015. Two additional CR-1000 dataloggers were used to record meteorological conditions: one at the subset of plots in the south-facing slope and one at the subset of plots with a west-facing slope. These dataloggers recorded hourly air temperature (\(^\circ\)C, 2 m above soil surface), soil temperature (\(^\circ\)C, 10 cm below soil surface), and soil water content (10 cm below soil surface).

**Aboveground phenology**

To estimate the timing of seasonal leaf cover expansion, canopy NDVI (normalized difference vegetation index) was recorded in each plot once per week from 2014-2015. NDVI =
(R800-R660)/(R800+R660), where R800 is the reflectance at 800nm, and represents a near infrared wavelength, while R660 is reflectance at 660nm and represents a photosynthetically active wavelength (as in Boelman et al., 2003). NDVI was recorded with a Unispec-DC (PP Systems, Haverhill, MA USA) and determined from comparisons of incident and reflected light in each plot. To account for light conditions each day, we calibrated the Unispec-DC with a white standard. Each measurement was taken with the Unispec-DC placed 2m above each plot to yield measurement footprint of 0.39m². Three measurements were averaged over each plot.

Statistical analyses

Because we were interested in timing of root growth, we wanted to remove the normal high spatial variation in amount of roots from minirhizotron tube to tube and plot to plot. Thus, we standardized data to the maximum value for that plot, summed over the two minirhizotron tubes, in that year. The proportion of maximum root production on a given date was computed by: root production on that date divided by maximum cumulative root production occurring in that plot during that year. We obtained the proportion of maximum NDVI and the proportion of maximum root standing crop for each plot in the same way.

To examine seasonal differences between warming treatments and vegetation types in volumetric soil water content, soil temperature, proportion of maximum root production, proportion of maximum root standing crop, and proportion of maximum NDVI, each of these variables was analyzed with a mixed model using time, treatment, vegetation type, time*treatment, and time*vegetation type as fixed effects. Plot nested within date was included as a random effect due to non-independence of plots measured repeatedly through time. Data were arcsine or square root transformed to achieve normality. All analyses were performed in SAS JMP Pro 10.0.2 (2012, Cary, NC).
Results

Effects of open top chambers

Across all dates and vegetation types, OTCs increased the average plot-surface maximum daily air temperature by 2.2±0.1 °C (mean±SE; Figure 1A; \( F_{1,1364}=68.1, P<0.001 \)). The OTC treatment was significant for all vegetation types (graminoids: \( F_{1,366}=90.4, P<0.001 \); mixed: \( F_{1,508}=16.9, P<0.001 \); shrub: \( F_{1,486}=4.5, P=0.03 \)) The graminoid plots experienced the largest increase in maximum air temperature at 4.7±0.2 °C, mixed plots experienced an increase of 1.7±0.1 °C, and shrub plots underwent the most modest warming, at 0.97±0.1 °C. OTCs had little effect on minimum daily air temperature (Figure 1B; increase of 0.11±0.04 °C; \( F_{1,1364}=0.95, P=0.33 \)). Between June and August 2015, warming advanced cumulative air temperatures by 40 growing degree-days.

Warmed plots had significantly cooler and drier soils than ambient plots (soil water content: \( F_{1,1007}=4.3, P=0.04 \); temperature: \( F_{1,954}=11.5, P<0.001 \)) but the mean effect size was modest. Averaged across all plots and depths, ambient and warmed plots only differed by 0.3 °C and soil water content of 0.011. Findings from the continuous dataloggers further suggest that there was very limited difference between treatments with measures of soil temperature and moisture nearly indistinguishable (Supplement 1).

Soil water content and temperature differed significantly between the three vegetation types (soil water content: \( F_{2,1004}=7.5, P<0.001 \); temperature: \( F_{2,956}=47, P<0.001 \)). On average (±SE), shrub plots were coolest (3.6±0.1 °C), grass plots were intermediate (4.1±0.1 °C), and mixed plots were warmest (4.7±0.1 °C). Shrubs plots were driest (soil water content: 0.25±0.003), mixed plots were intermediate in terms of soil moisture (0.31±0.004), and graminoid plots were the wettest (soil water content of 0.33±0.004).
Aboveground phenology

Although open top chambers warmed maximum air temperatures by an average of 2.2 °C, warming did not significantly alter the timing of aboveground growth, measured as relative NDVI (Figure 2; warming*date: $F_{22,905}=0.96$, $P=0.52$), although warmed plots peaked slightly (and non-significantly) earlier in the year in 2014. Vegetation types differed in the timing of aboveground leaf cover (Figure 2; vegetation type*date: $F_{44,905}=13.4$, $P<0.001$), with graminoids reaching peak leaf cover later than shrubs or mixed plots. There was no significant interaction between vegetation type and warming treatment in the timing of aboveground growth (warming*vegetation type*date: $F_{44,905}=1.3$ $P=0.08$).

We had large statistical power to determine a true difference in the means of NDVI between warmed and ambient plots. Our power to determine a 5% difference in the means was 0.78 (78% of the time we would correctly reject the null hypothesis if we reran the study many time with random samples), and our power to detect a 10% difference in the means was 0.99.

Root phenology

New root production was highest in spring (May and June) and in fall (late August to early September), and in 2014 there was a third peak in early July (Figure 3). The timing of root production differed by vegetation type, but not by warming treatment (Figure 3; warming*date: $F_{29,1250}=0.63$, $P=0.93$; Figure 3; vegetation type*date: $F_{58,1250}=3.1$, $P<0.001$). There was no significant interaction between vegetation type and warming treatment in the timing of growth (warming*vegetation type*date: $F_{58,1250}=0.53$ $P=0.99$).

Given high variation in root dynamics among plots and years, we had only modest power to determine a true difference in the means of root production between warmed and ambient plots. The power to determine a 25% difference in the means was 0.29, the power to detect a 50%
difference in the means was 0.80, and the power to detect a 75% difference in the means was 0.99.

In all years, relative root standing crop was highest late in the growing season. The timing of relative root standing crop differed by warming treatment and vegetation type (Figure 4; warming*date: $F_{28,1098}=2.12 \; P<0.001$; vegetation type*date: $F_{56,1098}=2.25 \; P<0.001$), but this was only significant in 2014. The mean difference in root standing crop in 2014 between ambient and warmed plots was 21±0.03%. Despite a significant time*treatment interaction in this year, the warmed and ambient treatments still peaked around the same date (September 6, the last sampling date in 2014).

**Discussion**

Experimental daytime warming of approximately 2°C did not generally alter above- or belowground phenology of graminoids or a shrub species in an Arctic ecosystem over two years aboveground and three years belowground. Many studies suggest that temperature is the primary control on plant phenology and associated carbon fluxes (Wielgolaski, 1999; Peñuelas and Filella, 2001). We suggest, however, that advances in phenology may not be driven primarily by temperature in all ecosystems, and terrestrial biosphere models with high temperature sensitivities may overestimate future carbon uptake in some ecosystems. Other factors, such as CO₂ concentration, soil nutrient availability, and timing of snowmelt may also be important drivers of aboveground phenology (Chapin et al., 1995; Iler et al., 2013; Sharp et al., 2013; Reyes-Fox et al., 2014). Plants in this ecosystem may rely on several cues concurrently in order to begin growth when conditions are most favorable.

The open top chambers (OTCs) advanced growing degree days by 40 days between June and August 2015 (assuming a growth threshold of 10°C), and we expected an associated
advancement in plant phenology. We had strong statistical power to detect differences in the timing of leaf cover (power of 0.8 to detect a 5% difference in the means), although our power to detect differences in the timing of root phenology was more modest, given inherent variation in root phenology (Radville et al, in review; power of 0.8 to detect a 50% differences in the means). The OTCs in this experiment only warmed daytime temperatures, but daytime temperatures are likely to have a strong influence on phenology. For example, in a study of temperate trees, Fu et al. (2016) found that the impact of daytime temperatures on leaf unfolding was three times stronger than the impact of nighttime temperatures. Thus, although this study may have under estimated the impact of warming, we would still expect a shift in the timing of growth if temperature were the primary constraint on phenology at this site.

We expected shrub plots to be the most responsive to warming, due to their elevated meristems, but these plots had the smallest amount of warming (0.97 °C). Shrub phenology may have shifted with greater amounts of warming, but we believe we would have seen at least a modest shift if temperature were the primary phenological control. We expected graminoid plots to be less responsive to warming because meristems are at or below the soil surface. The warming treatment only minimally impacted soil temperatures (0.3 °C difference between treatments), so it is possible that graminoid phenology may shift with future warming if soil temperatures are elevated.

Previous work near this study site reported advanced phenology with warming by OTCs (Post et al., 2008; Radville et al., 2016), but we did not find comparable advances in phenology in this study. In Post et al. (2008), distinct phases of phenology, such as leaf opening, flower set, and bloom, were recorded at one- to two-day intervals, rather than total leaf cover over the entire plot, as in this study. Because our metric of aboveground phenology was based on leaf cover and our temporal resolution was coarser (weekly), we may have missed fine scale changes in the timing in vegetation and of other events, such as the timing of reproductive growth. Warming effects in
both Post et al. (2008) and Radville et al. (2016) may have been due to a community shift to species that emerge earlier, as warmed plots moved from a graminoid-dominated to a shrub-dominated community after five years (Post and Pedersen, 2008). As well, a recent analysis of long-term observational data from this site indicated no advance in timing of leaf-out by *Salix glauca* shrubs, and only a very modest advance in the timing of leaf-out by *Betula nana* shrubs, in response to spring warming since 2002 (Post et al., in revision). In studies by Post et al., the temperature increase caused by OTCs was not directly measured, but because methods were very similar, we do not expect that warming was significantly greater in those studies.

Although there has been rapid warming of the Arctic in recent decades, the influence of warming on phenology may decrease with time (Kremers et al., 2015). Non-linear responses to warming with time were found in European tree species, as advances in leaf phenology were reduced by 40% over 33 years (Fu et al., 2015). It is possible that we observed a saturation effect of warming, wherein individual species do not continue to advance with additional warming (sensu Kremers et al. 2015).

The influence of warming may differ by latitude, as some evidence suggests a stronger influence of temperature at high latitudes (IPCC, 2014). A meta-analysis found that, on average, passive warming from one to four years advanced phenology in arctic and alpine ecosystems (Arft et al., 1999), but leaf phenology was advanced in three out of four years in the high Arctic, whereas leaf phenology was only advanced in one out of four years in the low Arctic. Experimental studies comparing the high and low Arctic suggest that temperature may be a stronger constraint at more northern sites and other factors, such as nutrient availability, may be stronger controls at more southern sites (Havström et al., 1993; Wookey et al., 1993). Because our site is in southern Greenland at a relatively warm, nutrient-poor site, temperature may not be the primary limiting factor. The lack of a treatment effect may also have been caused by a
relatively short period of warming (five years), although other studies describe advanced phenology after fewer years (Arft et al., 1999).

In order to predict the future carbon budget, it is important to understand constraints on plant phenology. In southwestern Greenland, observed advances in phenology have increased ecosystem carbon sink strength by an estimated 1.3 g C m\(^{-2}\) y\(^{-1}\) in Betula nana and 2.1 g C m\(^{-2}\) y\(^{-1}\) in graminoid tundra (Cahoon et al., 2016b). If warming is not the primary control on phenology, increased temperatures may not cause such strong carbon sinks. In conclusion, we suggest that temperature may not be the primary control on above- or belowground phenology in all arctic ecosystems, and factors such as water and nutrient availability should also be considered when making predictions about future phenological shifts.

**Acknowledgements**

This work would not have been possible without the work of Thomas Adams, Lissette Bencosme, Thomas Bentley, Eva Beyen, Sean Cahoon, Elizabeth Elmstrom, Chénira Smith, Savannah Putnam, Tyler Tran, and Casey Yarbrough. Funding was provided through NSF Arctic Natural Sciences Program (ARC-110738) to EP and DME.
Figure 4-1: A. The difference between daily maximum air temperature in warmed plot and daily maximum temperature in ambient plots (C). B. The difference between daily minimum air temperature in warmed plot and daily minimum temperature in ambient plots (C). In both panels, values are averages across all vegetation types. On average across all years and vegetation types, open-top chambers warmed maximum daily temperatures by 2.2±0.1°C (mean±SE) but had little effect on minimum daily temperature. Error bars represent standard error of the mean.
Figure 4-2: Relative leaf cover (NDVI on a given date/maximum NDVI in that year) averaged for ambient and warmed plots in 2015 and 2016. Dashed gray line and gray points are the mean of ambient plots and solid black line and points are the mean of warmed plots. There was no significant effect of the warming treatment. Error bars represent standard error of the mean.
Figure 4-3: Timing of relative new root production (root production on a given date/maximum root production in that year) in warmed and ambient plots for three growing seasons. The warming treatment did not have a significant effect on the timing of root production. Gray line and points are the mean of ambient plots and black line and points are the mean of warmed plots. Error bars represent standard error of the mean.
Figure 4-4: Timing of relative root standing crop (roots present on a given date/maximum root standing crop in that year) in warmed and ambient plots for three growing seasons. Gray line and points are the mean of ambient plots and black line and points are the mean of warmed plots. Error bars represent standard error of the mean.
References


mask variation in the direction and magnitude of short-term phenological shifts. American
Journal of Botany, 100(7): 1398-1406.

Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report
of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York,
NY, USA: Cambridge University Press, 1132 pp.

Kremers, K. S., Hollister, R. D., and Oberbauer, S. F., 2015: Diminished response of
arctic plants to warming over time. PLoS ONE, 10(3): e0116586.

McBean, G., Alekseev, G., Chen, D., Førland, E., Fyfe, J., Groisman, P. Y., King, R.,
Melling, H., Vose, R., and Whitfield, P. H., 2005: Arctic climate: past and present. In Symon, C.,
Arris, L., and Heal, B. (eds.), Arctic Climate Impacts Assessment (ACIA). Cambridge:
Cambridge University Press, 20-60.

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kübler, K.,
Bissolli, P., Braslavská, O. g., Briede, A., Chmielewski, F. M., Crepinsek, Z., Curnel, Y., Dahl,
Å., Defila, C., Donnelly, A., Filella, Y., Jatczak, K., Mâge, F., Mestre, A., Nordli, Ø., Peñuelas,

793-795.
Post, E. and Forchhammer, M. C., 2008: Climate change reduces reproductive success of an Arctic herbivore through trophic mismatch. Philosophical Transactions of the Royal Society B-Biological Sciences, 363(1501): 2369-2375.


Limited influence of root phenology on seasonal carbon exchange in Arctic shrub and graminoid tundra

Laura Radville¹, Thomas S. Adams¹, Patrick F. Sullivan², Jeffrey M. Welker³, Eric Post⁴, and David M. Eissenstat¹

¹Department of Ecosystem Science and Management and the Ecology Graduate Program, The Pennsylvania State University, University Park, PA, 16802, USA
²Environment and Natural Resources Institute, University of Alaska Anchorage, Anchorage, AK, 99508, USA
³Department of Biological Sciences, University of Alaska Anchorage, Anchorage, AK, 99508, USA
⁴Department of Wildlife, Fish, & Conservation Biology, University of California, Davis, Davis, CA, 95616, USA

Abstract

The timing and duration of plant growth is a strong indicator of seasonal patterns of carbon fluxes. Although the link between leaf phenology and ecosystem productivity has been documented, the link between root phenology and ecosystem respiration is less clear. Because roots can comprise over 75% of plant biomass in tundra ecosystems, this relationship may be particularly important in the Arctic. We examined the influence of leaf phenology and root phenology on carbon fluxes in shrub and graminoid communities in southwestern Greenland. We found that leaf phenology was strongly correlated with gross ecosystem production (carbon
uptake from atmosphere), but root phenology was not a strong control on ecosystem respiration (carbon release to atmosphere). Additionally, shrubs had less annual root turnover than either graminoids or mixed graminoid-shrub plots, so as shrub cover increases across the Arctic, there may be less belowground carbon allocation. This research suggests that leaf phenology, which is easier to track than root phenology, may be a good indicator of ecosystem respiration. Labor-intensive tracking of root phenology may not be necessary to predict the timing of ecosystem respiration.

Introduction

Patterns of root production and turnover are important drivers of plant resource uptake and carbon allocation belowground. The link between aboveground growth and ecosystem productivity is well documented (Richardson et al., 2013); however, the link between root production and turnover to ecosystem production is less well studied. This link is important, as root production can account for 8-67% of total net primary productivity and root traits may drive ecosystem processes (Grier et al., 1981; Keyes & Grier, 1981; Jackson et al., 1997; Bardgett et al., 2014). Root production may be a particularly strong flux in ecosystems where most plant biomass is belowground.

The Arctic has some of the highest root:shoot ratios in the world at about 5:1 (Mokany et al., 2006; Iversen et al., 2015). Understanding patterns of carbon allocation is important in the Arctic because recent warming has occurred at twice the global rate (McBean et al., 2005), and impacts of climate change may be stronger than in other ecosystems (Anisimov et al., 2007). The Arctic is an important sink for carbon dioxide and source of methane, but the total effect of warming on future carbon cycling in the Arctic is unclear (McGuire et al., 2009). Given high root biomass and the importance of root growth and turnover, belowground processes may be central
to understanding changes in ecosystem carbon exchange. Furthermore, shrubs are replacing graminoids as the dominant plant type in many regions of the Arctic (Myers-Smith et al., 2011). The conversion to shrub-dominated ecosystems could drastically change carbon cycling in the Arctic, as shrub-dominated ecosystems may have greater carbon uptake than graminoid communities (Sweet et al., 2015).

In this study, we examined the influence of vegetation type (shrub and graminoid) on above- and belowground phenology and carbon exchange. We tracked the phenology of graminoid communities (Poa spp.; Carex spp.) and patches of a shrub species (Betula nana) from 2014-2015. We also recorded net ecosystem exchange, gross ecosystem production, and ecosystem respiration in these ecosystems from 2014-2015 and estimated the influence of above- and belowground phenology on carbon fluxes. We expected roots to have a strong influence on ecosystem respiration, due to high root:shoot ratios reported in arctic ecosystems. The belowground growing season may be 50% longer than aboveground in the Arctic and is likely offset from the aboveground growing season, potentially due to soils warming slower than air in the spring and remaining warm later than air in fall (Abramoff & Finzi, 2015; Blume-Werry et al., 2015). Due to the offset between root and shoot phenology, we expected that carbon exchange would not be explained by shoot phenology alone and that root phenology may be a more important driver of ecosystem respiration during certain times of year. Given previous research (Sloan et al., 2016), we expected shrubs to have a greater asynchrony between root and shoot phenology than graminoids, and, therefore, that the timing of net ecosystem carbon exchange in shrubs would be less coupled to shoot growth than in graminoids.
Methods

This work was located at a Low Arctic study site near Kangerlussuaq, Greenland (67.11ºN, 50.30ºW). This area is composed of dry acidic tundra on noncarbonate bedrock in Arctic shrub-tundra (Elvebakk, 1999). The mean annual air temperature was 4.4°C in 2014 and -7.7°C in 2015. The experimental set-up for this site has been previously described in detail (Cahoon et al., 2016). Briefly, in 2012, 48 plots were established to compare grass and shrub phenology. Half of all plots (24 plots) were warmed by covering with 1.5m-wide cylindrical open-top chambers, following ITEX protocols (Henry & Molau, 1997). Of these plots, one third (16 plots) were in graminoid-only patches (*Poa* spp., *Carex* spp.), 16 plots were in shrub-only patches (*Betula nana*), and 16 plots were in “mixed” patches covered by approximately 50% *Betula* and 50% graminoids (n=8 per warming treatment by vegetation type). This site was well suited to comparisons between species due to small-scale patchiness of species distributions across the landscape. Warming did not significantly affect above- or belowground phenology in these plots and the influence of warming has been described in Chapter 4, so in this study we averaged the warming treatments in order to only compare vegetation types.

Aboveground phenology

To monitor aboveground phenology, NDVI (normalized difference vegetation index) was measured over each plot using a Unispec-DC (PP Systems, Haverhill, MA USA) each week from 2014-2015. We used NDVI = (R800-R660)/(R800+R660), where R800 is the reflectance at 800nm (a near-infrared wavelength), while R660 is reflectance at 660nm (a photosynthetically active wavelength). To account for daily light conditions, the Unispec-DC was calibrated to a white standard. Each measurement was taken 2m above the plot to obtain a measurement footprint of 0.39m². Three measurements were averaged per plot per day.
We did not directly measure aboveground biomass, but we estimated aboveground biomass using plot-level NDVI measurements and a published relationship between NDVI and aboveground biomass in the Arctic. We used the following relationship from Epstein et al. (2012): \( \text{NDVI} = 0.383 \ln(\text{biomass}) - 1.649 \), where biomass is in g m\(^{-2}\). This relationship was determined from thirteen total field sites from two transects: one in North America and one in Eurasia. Although neither transect included Greenland, the sites covered all five Arctic bioclimate subzones, and the relationship was strong \((R^2=0.94)\). For mixed and Betula nana plots, these estimates were similar to a previous study near this site that directly measured aboveground biomass (Pedersen & Post, 2008). Our graminoid estimates were higher than in Pedersen & Post (2008) because the NDVI-biomass relationship did not allow us to differentiate among vegetation types.

**Gas exchange**

\( \text{CO}_2 \) exchange was measured nearly weekly from May to September in 2014 in a subset of 16 plots \((n=4 \text{ per vegetation type [ambient plots only and shrub and graminoid only; no mixed plots]}))\). \( \text{CO}_2 \) exchange was measured nearly weekly from May to August in 2015 in a subset of 16 plots \((n=4 \text{ per warming treatment [warmed/ambient] by vegetation type [shrub/graminoid; no mixed plots]}))\). Measurements were recorded using a closed system LI-8100A (LI-COR Environmental, Lincoln, NE, USA) connected to a 75 × 75 × 75 cm clear acrylic chamber. To mix air in the chamber and monitor temperature and light, two small fans, a temperature probe, and a PAR (photosynthetically active radiation) sensor were installed inside the chamber. The chamber was sealed to the tundra using the approach outlined by Cahoon et al. (2012), wherein a plastic skirt was attached the bottom edges of the chamber and the skirt was sealed to the tundra with a heavy, metal chain. On each sampling date, we took two \( \text{CO}_2 \) flux measurements of 90s each at each plot. For the first measurement, the chamber was left open to the light, and this
served as a measure of net ecosystem exchange (NEE; negative values indicate net carbon uptake and positive values indicate net carbon release). We then lifted the chamber from the tundra to vent the air, placed a black cloth over the chamber to exclude light, and obtained a second measurement. The dark measurement represented ecosystem respiration (ER; positive values indicate carbon uptake). We excluded first 10 s of each measurement is order to eliminate noise associated with the chamber being placed on the plot. Gross ecosystem production (GEP) was assumed to be ER-NEE. The CO₂ concentrations were corrected for water vapor dilution inside the chamber (LI-8100A Manual, 2012).

**Root phenology and turnover**

To monitor root phenology, two minirhizotron tubes were installed in each plot in 2012. Minirhizotron tubes were clear acrylic tubes that were installed in the soil at a 30-degree angle to the vertical. Tubes were anchored to the soil with steel rods and shielded from weather, light, and solar radiative heating by sealing each tube with a plumbing plug, wrapping it with electrical tape, painting it white, and covering it with a white, aluminum radiation shield. The inside of the tube was filled with pipe insulation to minimize temperature changes. A minirhizotron camera (Bartz Technology) was used to take root images weekly from May-September in 2014 and May-August in 2015. The length of roots present on each date was quantified using root-tracing software Rootfly (Clemson University, Clemson SC). A root turnover coefficient was computed for each plot as annual root production (cm cm⁻²) viewed in minirhizotrons divided by the maximum root standing crop (cm cm⁻²) viewed in minirhizotrons in that year. Root phenology was tracked from May-June in 2016 to verify early season trends from 2014-2015 and is presented in Appendix Figure A1.

*Comparison of minirhizotrons to root density in soil*
In 2012, at the same time experimental plots were selected, an additional 24 plots were selected to serve as destructive plots to compare minirhizotron images to harvested root densities. These plots were the same size as the main study plots with the same focal plant species and two minirhizotrons installed in each of plots. Half of all plots were warmed with open top chambers, and they were divided between shrub, mixed, and graminoid plots (n=4 per warming by vegetation type).

On August 15, 2015, we used the minirhizotrons to assess root standing crop in the destructive plots just prior to obtaining three soil cores in each of these plots. Cores were 5.08 cm in diameter and as deep as the active layer allowed (from 26 to 81 cm). Soil cores were separated by 10-cm-depth increments, and roots were washed in the field and stored in plastic bags with paper towels in an approximately 10% ethanol solution. Obviously dead, black roots were discarded. Root samples were stored long-term at 3C. To process each sample, a random, representative subsample of roots was removed from the bagged core. This subsample was washed thoroughly and scanned using an EPSON Perfection 4490 Photo scanner. Total root length and average diameter of the subsample were analyzed using WinRhizo software (Regent Instruments, Inc.). Both the subsample and the rest of the roots from the core were dried for 24 hours in a drying oven at 60C and weighed to determine the dry root biomass. Root length density (cm cm\(^{-3}\)) was determined from root subsamples and was scaled up to the full sample, and root biomass (g m\(^{-2}\) ground) was determined from full core sample to the maximum depth acquired. Previous research from Barrow, Alaska suggests that 35% of tundra roots are alive at a given time on average (Dennis et al., 1978), so we multiplied root density by 0.35 in order to obtain an estimate of living root biomass. This likely gives a conservative estimate of living root biomass, as we had previously discarded obviously dead roots. Alternatively, decomposition is likely slower at our dry, acidic site, as compared to the moist tundra at Barrow, AK, so more roots in our samples may have been dead without showing obvious signs of decay yet. Additional caveats
to the estimation include that Dennis et al. (1978) conducted their study only visually assessing the percentage of living roots, and their study was conducted on a different plant community, composed primarily of graminoids (Dennis & Johnson, 1970). In another study, on Ellesmere Island in a mesic-xeric dwarf shrub heath, they visually sorted roots and estimated that about 21% of the root standing crop was living in the 1980s (Henry et al., 1990) and 13% was living at the same site in 2011 (Hill and Henry, 2011). Another study in the High Arctic on sedge moss meadow found that 52% of roots were living, based on visual estimates (Muc, 1977). Although none of these studies were done on the same plant community as in this study and all were assessed visually (rather than experimentally), the value of 35% chosen for this study is at least in mid-range of existing studies. Clearly, more studies are needed that experimentally determine the amount of root standing crop that is living at a given time.

Next, we estimated the Betula nana biomass that came from first through third order roots, which are most likely to be absorptive of water and nutrients (Guo et al., 2008). We assumed that all grass root biomass was absorptive. To determine the length and mass of the first three Betula root orders, two root systems were dug up from shrubs near the study site. Samples were washed in the field, placed in plastic bags, and stored long-term at 3C. To separate by root order, a sub-sample of intact, well-branched roots was removed. From this sub-sample, roots belonging to each of the first three orders were scanned using an EPSON Perfection 4490 Photo scanner. The scanned images were analyzed for root length and average diameter using WinRhizo. The roots were then placed in coin envelopes and dried for 24 hours in a drying oven at 60C. Dried roots were weighed to determine dry mass. From this procedure, we determined that Betula third order roots were approximately 0.28mm in diameter, so, in order to only analyze absorptive roots, we excluded all roots larger 0.28mm in all analyses. For Betula nana, 63% of the total root mass and 83% of total root length came from first through third order roots. For
mixed plots, 95% of total root mass and 98% of total root length came from first through third orders.

Living root biomass of first through third orders in cores was multiplied by the turnover coefficient (as determined from minirhizotrons, described above) to estimate root turnover in each year (g roots m\(^{-2}\) ground surface year\(^{-1}\)). Total living belowground biomass estimates were divided by estimated aboveground biomass (described above) in order to calculate root:shoot ratios at this site. Root length obtained from minirhizotrons in harvest plots was compared to root length and root biomass obtained from destructive harvests. These relationships between root length on minirhizotron tubes and root length density in soil cores were comparable, although minirhizotrons had many fewer roots visible (Appendix Figure A2).

Estimating contribution of root phenology to ecosystem respiration

We modeled ecosystem respiration using the model from Shaver et al. (2007): 
\[ ER = (R_0 * e^{\beta T} * LAI) + R_x, \]
where \( R_0 \) is leaf respiration at 0°C (\( \mu mol m^{-2} leaf s^{-1} \)), \( \beta \) is empirically fit from the data (°C\(^{-1}\)), \( T \) is air temperature (°C), and \( R_x \) is respiratory CO\(_2\) that is likely from deep soil horizons (\( \mu mol m^{-2} ground s^{-1} \)). In order to empirically determine \( R_0 \), \( \beta \), and \( R_x \), the model was fit to two thirds of the data (147 observations). Nonlinear regression in Excel Solver (Microsoft Excel 2015 v.15.14) was used to minimize the root mean-square error (RMSE) of predictions versus observations. The data for ER observations and \( T \) were obtained experimentally, and LAI was estimated from measured NDVI values using a previously published relationship determined at the same site (Cahoon et al., 2016). Estimated \( R_0 \) was 0.15 \( \mu mol m^{-2} leaf s^{-1} \), \( \beta \) was 0.11, and \( R_x \) was 2.1 \( \mu mol m^{-2} ground s^{-1} \). The \( Q_{10} \) temperature response was calculated using \( Q_{10} = e^{10\beta} \), and the result was 3.07. These values are similar to a previous study at this site (Cahoon et al., 2016). The average values across both graminoids and shrubs in Cahoon et al. (2016) were a \( Q_{10} \) of 3.21, \( R_0 \) of 0.24, \( \beta \) of 0.11, and \( R_x \) of 1.78. All respiration rates throughout the season were adjusted for
temperature using the \( Q_{10} \) and the relationship \( ER_2 = ER_1 \times Q_{10}((20 - T_1)/T_1) \), where \( ER_2 \) is the estimated respiration rate at 20C, \( ER_1 \) is the observed respiration rate, and \( T_1 \) is the air temperature when \( ER_1 \) was measured (Atkin & Tjoelker, 2003). To evaluate the model, we regressed the model estimates of temperature-adjusted (to 20C) \( ER \) with temperature-adjusted measurements of \( ER \) using the one third of data that was not used to cross-validate the model.

Once the model was constructed, we compared the error in the model (observed \( ER \) at 20C - estimated \( ER \) at 20C) to the new root production (cm cm\(^{-2}\) day\(^{-1}\)) and root standing crop (cm cm\(^{-2}\)) obtained from minirhizotrons on each date. If error in the \( ER \) model was due to the influence of root production or standing crop, we expected a positive relationship between model error and roots. We also compared error in the model to soil temperature at 10cm from the organic layer.

**Abiotic conditions**

To measure soil moisture in all plots, time domain reflectometry (TDR) sensors were permanently installed vertically from 10-20, 20-30, and 30-40cm from the bottom of the organic layer. Probes were not permanently installed from 0-10cm because a relocatable probe was used to measure soil moisture from 0-10 cm on each sampling date. To measure soil temperature, thermocouple probes were installed at 20, 30, and 40cm from the bottom of the organic layer and a relocatable probe was used to measure soil temperature at 10cm on each sampling date. Soil moisture and temperature were recorded at all depths in all plots on each date that root phenology was measured and to 10cm on dates that carbon exchange was measured. To track nearly continuous abiotic measurements, CR-1000 dataloggers (Campbell Scientific, Logan, UT) were installed in a subset of 12 plots (n=2 per warming by vegetation treatment). These dataloggers scanned air temperature (10cm from the soil surface), soil temperature (10cm from the bottom of
the organic layer), and soil moisture (0-10 cm from the organic layer) every 30 s and recorded hourly averages.

**Statistical analyses**

Because our primarily focus was shifts in timing of events, NDVI, new root production, and root standing crop were standardized to the maximum value for that plot during that year (e.g. root production on that date divided by maximum cumulative root production occurring in that plot during that year). This approach reduced variation among plots and years so that variation in timing of events was easier to detect. Relative NDVI and standing crop data were arcsine transformed, and relative production data were square root transformed to achieve normality. To examine seasonal differences between treatments and vegetation types in volumetric soil water content, soil temperature, carbon fluxes, proportion of maximum root production, proportion of maximum root standing crop, and proportion of maximum NDVI, each of these variables was analyzed with a mixed model using plot as a random effect repeated through time and using the following fixed effects: time, vegetation type, warming, vegetation type*time, warming*time, and warming*vegetation type*time. To examine the influence of patterns of NDVI and root phenology on NEE, ER, and GEP, we examined correlations between absolute root standing crop, absolute NDVI, and gas exchange (NEE, GEP, and ER) with Spearman’s ρ. These patterns were also separated by vegetation type and season where we defined “spring” as from 1 May through 30 June, “summer” as 1-30 July, and “fall” from 1 August until the final measurement that season on August 24. To examine relationships between ER and other environmental variables, all variables were z-score transformed and Pearson correlations were determined ER and all other variables. All statistical analyses were performed using SAS JMP Pro 10.0.2 (2012, Cary, NC).
Results

Above- and belowground phenology

Phenology differed by vegetation type above- and belowground (Figures 1 and 2; effect of vegetation type*date on root production: $F_{38,1256}=3.1, P<0.001$; effect of vegetation type*date on root standing crop: $F_{38,1105}=2.25, P<0.001$; effect of vegetation type*date on NDVI: $F_{44,905}=13.4, P<0.001$). In graminoids, root and shoot phenology were offset: root standing crop was lowest in mid-season, when leaf cover was highest. In shrubs, there was no visible trade-off between roots and leaves. Shrub leaf cover peaked early in the season and stayed high, and root standing crop steadily increased throughout the season.

Gas exchange

Vegetation types had significantly different patterns of gross ecosystem production (Figure 3; vegetation type*date: $F_{21,223}=2.6, P<0.001$) and net ecosystem exchange differed (Figure 3; vegetation type*date: $F_{21,238}=4.2, P<0.001$). Shrubs remained a source of carbon later into the early season than graminoids, but shrubs were also more of a carbon sink in mid summer. The timing of ecosystem respiration was only marginally different by vegetation type (Figure 3; vegetation type*date: $F_{21,239}=1.6, P=0.06$).

Correlations between gas exchange and phenology

In general, leaf phenology was more strongly correlated with carbon fluxes than root phenology (Figure 4; Table 1). On average for both vegetation types (graminoid and shrub), in spring and in fall there was no significant correlation between root standing crop and ER. NDVI was negatively correlated with GEP in both spring and fall. NDVI was positively correlated with ER in the spring and negatively correlated with ER in fall.
In both graminoids and shrubs, root standing crop was not correlated with ER. Because root and shoot phenology were asynchronous only in graminoids, we examined the seasons separately in graminoid plots. In spring in graminoid plots, ER was not significantly correlated with root standing crop but was positively correlated with NDVI. In fall in graminoid plots, ER was not significantly correlated with either leaf or root phenology.

**Contribution of root phenology to ecosystem respiration**

The Shaver et al. model of ER provided a poor fit to the observed ER measurements. The $R^2$ was 0.35, slope was 0.23, and the intercept was 2.3. Using only the portion of the data not used to estimate model parameters, the error in the ER model was not significantly correlated with root production rate or root standing crop (Figure 5A and 5B; $F_{1,73}=0.2, P=0.67$ and $F_{1,73}=1.1, P=0.3$, respectively). Error in the model was positively correlated with soil temperature (Figure 5C; $F_{1,71}=6.3, P=0.01$). At lower temperatures, the model tends to overestimate ER.

**Root turnover**

On average, 19±1% (mean of all plots ± SE) of the living root system was replaced each year, which equated to 363±27 g biomass · m$^{-2}$ · yr$^{-1}$ (of first through third order roots). This suggests that, assuming a steady-state, roots in this ecosystem live for over five years. Vegetation types did not have significantly different rates of root turnover ($F_{2,138}=0.5, P=0.63$; grass: 0.20±0.02 yr$^{-1}$ (mean±SE); mix: 0.18±0.01 yr$^{-1}$; shrub: 0.18±0.02 yr$^{-1}$). Root length density (length of roots per unit volume of soil; measured from 2015 harvest) of living and dead roots significantly differed between the three vegetation types (Figure 7; $F_{2,21}=6.6, P=0.006$), and graminoid plots had the highest densities (graminoids: 394±74 cm cm$^{-3}$; mixed: 354±66 cm cm$^{-3}$; and shrubs: 115±24 cm cm$^{-3}$). Because living root biomass of first through third order roots, as measured in the harvest, differed among vegetation types (Figure 6B; $F_{2,21}=18.9, P<0.001$;
graminoids: 1744±173 g m$^2$; mixed: 2785±356 g m$^2$; and shrubs: 742±93 g m$^2$), the amount of annual root biomass turnover also differed (Figure 6A; $F_{2,90}=21.1, P<0.001$). Mixed plots had the highest turnover rate (680±59 g roots $\cdot$ m$^{-2} \cdot$ year$^{-1}$), turnover in graminoids was intermediate (503±57 g roots $\cdot$ m$^{-2} \cdot$ year$^{-1}$), and shrubs had the smallest turnover rate (221±27 g roots $\cdot$ m$^{-2} \cdot$ year$^{-1}$). The maximum estimated aboveground biomass in 2015 differed slightly by vegetation type, but was only marginally significant (Figure 6C; $F_{2,23}=3.3, P=0.055$). Shrubs had the highest aboveground biomass (384±36 g m$^2$), mixed plots had intermediate biomass (347±19 g m$^2$), and graminoids had the least (293±16 g m$^2$).

**Abiotic conditions**

Soil water content and temperature averaged from 0-40 cm differed significantly among the three vegetation types (soil water content: $F_{2,1004}=7.5, P<0.001$; temperature: $F_{2,950}=47, P<0.001$). On average (±SE), shrub plots were coolest (3.6±0.1°C), grass plots were intermediate (4.1±0.1°C), and mixed plots were warmest (4.7±0.1°C). Shrubs plots were driest (soil water content: 0.25±0.003), mixed plots were intermediate (0.31±0.004), and graminoid plots were the wettest (soil water content of 0.33±0.004).

**Discussion**

Despite high root:shoot ratios (Figure 8), ecosystem respiration was driven more by leaf phenology than by root phenology (Figure 4; Table 2). Additionally, error in an ecosystem respiration model could not be attributed to root production rate or to root standing crop (Figure 5). We expected root production and standing crop to play a larger role in ecosystem respiration, given that belowground biomass was much larger than aboveground biomass. Our estimated root:shoot biomass estimates are on the high end of the range found in published literature.
(Figure 8; Jackson et al., 1996; Mokany et al., 2006; Iversen et al., 2015). For example, Iversen et al. (2015) reported a median root:shoot ratio of 2.3 for grasses and 1.7 for deciduous shrubs, and our median values were 6.2 for grasses and 3.1 for shrubs. Mokany et al. (2006) reported a mean root:shoot ratio of 4.8 ± 1.2 (± SE) and range of 1.2 to 15 for all tundra vegetation, and the mean ratio in this study was 6.4 ± 0.61 and range was 1.8 to 13.

Ecosystem respiration may not be driven by presence of roots if root respiration is controlled by factors other than root growth rate and root presence. The influence of leaf phenology on carbon fluxes in this study suggests that photosynthate is a strong control on respiration at this site as well. This is supported by Figure 4D and Table 2, which show that ecosystem respiration was significantly correlated with seasonal leaf cover. Root respiration may be driven primarily by availability of photosynthate (in conjunction with temperature) as was found in studies of a boreal pine forest and a temperate forest (Hogberg et al., 2001; Janssens et al., 2001; Curiel Yuste et al., 2004). Janssens et al. (2001) posit that productivity drives autotrophic respiration through carbon allocation from leaves and drives heterotrophic respiration because microbes preferentially decompose new litter (Schimel et al., 1994). Another study found that heterotrophic respiration at this site accounted for about 53% of total soil respiration, and they suggest that both autotrophic and heterotrophic respiration rely on current photosynthate to fuel respiration (Cahoon et al., 2016).

Although root phenology did not explain error in the ecosystem respiration model, alternate sources of unexplained error could include soil temperature and lag effects related to litter fall. Root respiration may be limited, in part, by soil temperature (Boone et al., 1998; Huang et al., 2005), and heterotrophic respiration may be even more sensitive to temperature than roots (Bhupinderpal et al., 2003). In support of this, we found a positive relationship between soil temperature and model error, suggesting that soil temperature, through its effect on autotrophic and heterotrophic respiration, plays a role in ecosystem respiration at this site not captured in
current model parameters (Figure 5C). At low soil temperatures, the ER model over-estimated respiration. This may be due to the fact that root respiration is limited at low soil temperatures, and the model cannot account for reduction in respiration associated with low soil temperature. The $Q_{10}$ temperature adjustment accounts for differences due to air temperature, but not does account for difference due to soil temperature.

Because root and leaf phenology were asynchronous in graminoids, we expected roots to contribute more to ecosystem respiration during periods when root standing crop was large but leaf cover was small. This was not the case, however, likely due to the small influence of root presence on ecosystem respiration. Respiration of existing roots may be highly constrained by the availability of photosynthate and by soil temperature. In graminoids, root standing crop was highest in spring and fall, when leaf cover was low (Figure 1). In spring, however, ER was only correlated with NDVI and not root standing crop. Over the entire growing season, graminoid leaf cover was strongly correlated with carbon exchange (negatively correlated with NEE and GEP, and positively correlated with ER), but root phenology was not correlated with ER and was moderately negatively correlated with NEE. In shrubs there was no asynchrony between leaf and root phenology, as both leaf cover and root standing crop were high in mid-season (Figure 1). Root phenology was not correlated with ER in shrubs, but leaf phenology was strongly correlated with ER and GEP.

A previous study in the sub-Arctic found a stronger asynchrony between root and shoot phenology in shrubs than in a sedge-dominated community (Sloan et al., 2016), which contrasts with our findings. Our results may have differed because we analyzed the timing of leaf presence, rather than the timing of new leaf production, as in their study. Although we did not collect data on the timing of new leaf production, our root production data also support a stronger synchrony between roots and leaves in shrubs. It is possible that the asynchrony may differ between species
even within a vegetation type, or that ecosystem differences in abiotic environmental factors drive differences in phenology.

Although differences in root phenology between the shrubs and graminoids did not have a strong influence on ecosystem carbon fluxes, vegetation differences in root turnover may influence yearly carbon uptake and belowground carbon storage. Graminoids and shrubs had strong differences in annual root turnover, and shrubs had lower annual root biomass turnover than either mixed or graminoid plots (Figure 6A). Mixed plots had 1.6 times higher root biomass than graminoids and 3.8 times higher root biomass than shrubs, so annual root turnover was 1.4 to 3.1 times higher in mixed plots than either vegetation type alone (Figure 6A). Although we do not know the exact cause of higher root biomass in mixed plots, it may be the result of interspecific competition. Each vegetation type may be producing more roots in these ecotones in order to obtain nutrients that would otherwise be taken up by the other vegetation type.

Less root turnover results in a smaller amount of net primary production allocated belowground for root growth (Matamala et al., 2003). Therefore, as shrub cover increases across the Arctic, there may be less carbon allocation to roots at the ecosystem-level, and as a result, less belowground carbon storage. In the advancing ecotone where shrubs meet graminoids, however, there may be high levels of carbon allocation to roots to support large amounts of root biomass turnover.

While some investigators argue that root traits drive ecosystem processes (Bardgett et al., 2014), we found mixed evidence for this perspective in an arctic system from root measurements. Despite high root:shoot ratios in the Arctic, root phenology may not be a strong control on ecosystem respiration. In general, we found that leaf phenology was more strongly correlated with net ecosystem exchange. Leaf phenology, which is much easier to track than roots and can be viewed remotely, may be a good indicator for the timing of carbon fluxes in the Arctic. In addition, the increase of shrubs across the Arctic may result in lower root densities and carbon
allocation belowground, which could reduce total soil respiration. In agreement with previous research showing an asynchrony between above- and belowground growth in many ecosystems, we found a strong asynchrony between root and shoot phenology in graminoids. This suggests that methods that track phenology based on leaf growth alone may underestimate the amount of plant production that occurs early and late in the season, even if these differences only weakly influence ecosystem respiration.

Acknowledgements: We are deeply grateful for assistance from Lissette Bencosme, Thomas Bentley, Eva Beyen, Sean Cahoon, Elizabeth Elmstrom, Chénira Smith, Savannah Putnam, Tyler Tran, and Casey Yarbrough. Funding was provided through NSF Arctic Natural Sciences Program (ARC-110738) to EP and DME and an ARCS fellowship to LR.
Figure 5:1: The proportion of maximum root standing crop and leaf cover in 2014 and 2015, measured as NDVI, in graminoids (A), mixed graminoids and shrubs (B), and shrubs (C; Betula nana). Dotted gray line represents proportion of maximum root standing crop (root standing crop observed/maximum root standing crop in that year). Solid black line represents proportion of maximum NDVI (NDVI on that date/maximum NDVI in that year). Error bars indicate ±1 standard error of the mean (N=16).
Figure 5-2: Relative new root production (new roots produced / day on that date/ maximum new roots produced / day in that season) from 2014 and 2015 in graminoids, mixed graminoids and shrubs, and shrubs (Betula nana). Solid gray lines represent graminoid plots, solid black lines represent shrub plots, and dotted black lines represent mixed plots. Error bars indicate ±1 standard error of the mean (N=16).
Figure 5-3: Carbon exchange in 2014 and 2015 in graminoid and shrub (Betula nana) plots, averaged for warmed and ambient treatments. Solid lines with filled black circles are shrub plots, and dashed lines with unfilled squares are graminoid plots. Gross ecosystem production (GEP) represents the net CO$_2$ produced (GEP = NEE – ER; negative values indicate carbon uptake), net ecosystem exchange (NEE) is net CO$_2$ exchange of the ecosystem at mid-day, and ecosystem respiration is the net CO$_2$ flux to the atmosphere (ecosystem carbon exchange in darkness). Negative values on the y-axis below the solid line indicate net CO$_2$ uptake by ecosystem, and positive values above the solid line indicate net CO$_2$ release to the atmosphere. Error bars represent ±1 standard error of the mean (N=8).
Figure 5-4: A. Lack of significant correlation between ecosystem respiration (ER) at 20C and live root production. B. Lack of significant correlation between ER at 20C and live root standing crop. C. Significant correlation between GEP at 20C and NDVI. D. Significant correlation between ER at 20C and NDVI. Dashed lines are derived from mixed models. Below the solid line there is a net CO₂ uptake by ecosystem, and above the solid line there is a net CO₂ release into the atmosphere.
Figure 5-5: A. The relationship between error in an ecosystem respiration (ER) model (observed ER - estimated ER) and root standing crop. The relationship is not significant. B. The relationship between ER model error and root production rate. The relationship is not significant. C. The significant relationship between ER model error and soil temperature at 10cm. The dashed line represents the results of a mixed model of error and soil temperature, with plots repeated through time.
Figure 5-6: **A.** Root turnover from 2014-2015, separated by vegetation type (graminoids, mixed plots, and *Betula nana* shrub plots). Root turnover is estimated for first through third order, absorptive, roots. **B.** Live root biomass (g m\(^{-2}\)) of first through third order roots estimated from harvest measurements in 2015. **C.** Aboveground biomass (g m\(^{-2}\)) estimated from relationship between measured NDVI in 2015 and published relationship between NDVI and shoot biomass. Black bars represent graminoid plots, dotted bars represent mixed plots, and gray bars represent shrub (*Betula nana*) plots. Error bars represent ±1 standard error of the mean (N=8).

Figure 5-7: Root length density of *Betula nana*, graminoids, and mixed plots in 2015, separated by depth. Gray bars are the mean of graminoid plots, striped bars are the mean of mixed plots, and black bars are the mean of *Betula* plots. Error bars represent the standard error of the mean (N=8).
Root:shoot ratio

<table>
<thead>
<tr>
<th></th>
<th>Graminoids</th>
<th>Mixed</th>
<th>Shrubs</th>
<th>Grasses</th>
<th>Deciduous shrubs</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iversen et al. 2015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5-8: Comparison of our estimates of live root:shoot ratios to those from review of other tundra studies by Iversen et al. (2015). Root:shoot ratios in this study are generated from total live root biomass (all root orders) and aboveground biomass estimated from measured NDVI. Center line represents median, upper limit of box is 75th percentile, lower limit of box is 25th percentile, and error bars represent 10th and 90th percentiles (N=8 in this study).
Table 5-1: Seasonal correlations (Spearman’s ρ) between plant phenology and carbon fluxes in 2014 and 2015. Leaf phenology is measured as NDVI on each date, and root phenology is root standing crop on each date. Gross ecosystem production (GEP) is CO₂ production (GEP = NEE – ER), net ecosystem exchange (NEE) is net CO₂ exchange at mid-day, and ecosystem respiration is net CO₂ flux to the atmosphere. *P<0.05; **P<0.01; ***P<0.001; ns, not significant.

<table>
<thead>
<tr>
<th>Vegetation type</th>
<th>Season</th>
<th>Plant organ</th>
<th>NEE</th>
<th>GEP</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Betula nana and graminoids, average</em></td>
<td>All, average</td>
<td>Roots</td>
<td>-0.1*</td>
<td>-0.1*</td>
<td>+0.04 (ns)</td>
</tr>
<tr>
<td></td>
<td>All, average</td>
<td>Leaves</td>
<td>-0.6***</td>
<td>-0.6***</td>
<td>+0.2***</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>Roots</td>
<td>-0.2*</td>
<td>-0.2*</td>
<td>+0.1 (ns)</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>Roots</td>
<td>-0.08 (ns)</td>
<td>+0.009 (ns)</td>
<td>-0.04 (ns)</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>Leaves</td>
<td>-0.7***</td>
<td>-0.6***</td>
<td>+0.4***</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>Leaves</td>
<td>-0.4***</td>
<td>-0.3*</td>
<td>-0.3*</td>
</tr>
<tr>
<td><em>Betula nana</em></td>
<td>All, average</td>
<td>Roots</td>
<td>-0.2*</td>
<td>-0.1 (ns)</td>
<td>-0.05 (ns)</td>
</tr>
<tr>
<td><em>Graminoids</em></td>
<td>All, average</td>
<td>Roots</td>
<td>-0.2*</td>
<td>-0.1 (ns)</td>
<td>-0.04 (ns)</td>
</tr>
<tr>
<td></td>
<td>All, average</td>
<td>Leaves</td>
<td>-0.5***</td>
<td>-0.5***</td>
<td>+0.3**</td>
</tr>
<tr>
<td></td>
<td>All, average</td>
<td>Leaves</td>
<td>-0.5***</td>
<td>-0.6***</td>
<td>+0.4***</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>Roots</td>
<td>-0.3*</td>
<td>-0.2*</td>
<td>+0.1 (ns)</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>Leaves</td>
<td>-0.7***</td>
<td>-0.6***</td>
<td>+0.4**</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>Roots</td>
<td>-0.2 (ns)</td>
<td>-0.1 (ns)</td>
<td>-0.2 (ns)</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>Leaves</td>
<td>-0.3 (ns)</td>
<td>-0.4*</td>
<td>+0.3 (ns)</td>
</tr>
</tbody>
</table>

Correlation between ER and the following variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDVI</td>
<td>0.25 ***</td>
</tr>
<tr>
<td>GEP</td>
<td>-0.47 ***</td>
</tr>
<tr>
<td>Soil temperature at 10cm</td>
<td>0.29 ***</td>
</tr>
<tr>
<td>Air temperature at 10cm</td>
<td>0.12 (ns)</td>
</tr>
<tr>
<td>NEE</td>
<td>-0.11 (ns)</td>
</tr>
</tbody>
</table>
Root production 0.11 (ns)
Soil water content -0.082 (ns)
Root standing crop 0.042 (ns)

Table 5-2: Seasonal correlations (Pearson) between ecosystem respiration and other environmental variables in 2014 and 2015. NDVI is normalized difference vegetation index, Gross ecosystem production (GEP) is CO₂ production (GEP = NEE – ER), net ecosystem exchange (NEE) is net CO₂ exchange at mid-day, and ecosystem respiration is net CO₂ flux to the atmosphere. *P<0.05; **P<0.01; ***P<0.001; ns, not significant.

References


Chapter 6

Synthesis and future directions

Plant phenology of the timing of life history events, such as leaf emergence, flowering and leaf senescence, is an important driver of many ecosystem processes (Richardson et al., 2013). Aboveground plant phenology is one of the strongest indicators of climate change (IPCC, 2014), because there is a clear link between air temperature and leaf emergence (Wielgolaski et al., 2011). The influence of warming on root phenological events, such as root growth initiation and peak root growth, however, is not well understood, as current studies provide conflicting conclusions. If root phenology does not advance with warming, advances in aboveground growth with climate change may not match whole-plant temporal shifts. This mismatch may be particularly important to consider in ecosystems with high root:shoot ratios, such as tundra (Mokany et al., 2006).

One reason drivers of root phenology are particularly hard to determine is due to a lack of quantitative analyses in current studies, as outlined in chapter one. Only 29% of currently published root phenology studies quantitatively analyzed the timing of growth. Additionally, previous studies do not often separate root phenology by different times of year, as is typically done aboveground (such as leaf-out, bud-burst, and leaf senescence). Drivers of root phenological events such as root initiation, peak root growth rate, and root growth cessation may shift, and thus should be analyzed separately in future studies. This view is supported in chapter two, where we quantitatively analyzed two long-term datasets of root phenology in grape and separately analyzed root initiation, peak root growth rate, and root growth cessation. The drivers of growth differed among these temporal phases, confirming that drivers of root phenological events change throughout the year.
Another reason for a lack of understanding of root phenology is the paucity of long-term studies. In chapter two, root phenology was monitored over three and five years, and this represents one of the longest-term published root phenology datasets. When searching the literature for other studies that examined both above- and belowground phenology concurrently for at least two years, there were only eight studies that met these criteria, and none were longer than four years. Multi-year datasets are particularly valuable because, in chapter two, we found that root phenology was more than twice as variable among years as aboveground phenology. This variation may make it particularly difficult to determine drivers of root growth. We also believe this variation is the reason we were not able to detect a strong link between aboveground phenology and root phenology, which means we are not able to predict the timing of root growth using broad aboveground phenological phases. High variation, limited linkages with aboveground phenology, and a paucity of long-term data make it difficult to determine what controls root phenology. Despite these limitations, future experiments that track long-term aboveground and belowground phenology, while concurrently tracking abiotic conditions, may elucidate understand drivers of root growth in different ecosystems.

These types of studies may be particularly important in the context of climate change. If warming advances the timing of aboveground growth but not the timing of belowground growth, the timing of carbon acquisition may be uncoupled from the timing of root production and nutrient acquisition. We investigated these ideas in chapter three, where we used a long-term warming site to investigate how increased air temperature and herbivore exclusion affect root and leaf phenology. We found that, although warming advanced the timing of leaf phenology and herbivore grazing delayed leaf phenology, neither treatment significantly impacted the timing of belowground growth. This suggests that increased air temperatures with climate change may not cause root phenology to advance in the same way as leaf phenology. These findings are important because aboveground phenology is used to track whole-plant phenology and predict the timing of
carbon exchange, but if roots do not advance with warming, shifts in whole-plant phenology with warming may be more modest than predicted. This is an especially important consideration for climate models, which assume that roots and leaves respond to warming in the same way and that their growth occurs at the same time (Abramoff and Finzi, 2015).

These climate models may also inaccurately predict the influence of climate change because warming may not influence all ecosystems in the same way. In chapter four, we show that warming of air temperature about two degrees Celsius did not advance above- or belowground phenology in dry acidic tundra in the Low Arctic. This ecosystem may be constrained more by other factors, such as the timing of nutrient and water availability, rather than by temperature. Although we know that the Arctic is warming at about twice the global rate (McBean et al., 2005), this may not advance the timing of plant growth and, thereby, the timing of carbon fluxes. Current models may overestimate the advance in the timing of gross ecosystem production and associated carbon uptake. If the influence of warming on plant growth is not consistent across ecosystems, ecosystem modeling efforts may need to be considerably more contextual to accurately predict carbon uptake.

In chapter five, we suggest that simple models, which only consider aboveground growth and not root growth, may be adequate for predicting carbon exchange in an Arctic system. We found that root phenology was not significantly correlated with ecosystem respiration, and the error in an ecosystem model could not be attributed to root phenology. These results show that, even in ecosystem where most plant biomass is belowground, adding root phenology to ecosystem models is not likely to improve estimates of carbon exchange. This makes modeling efforts considerably easier, given that aboveground phenology is much easier to track, and remotely sensed leaf phenology data are widely available.

Overall, this dissertation advances current understanding of root phenology by outlining ways future studies can be improved, such as by implementing long-term studies, partitioning the
belowground growing season into discrete phenological phases, and by experimentally separating abiotic factors in order to determine which ones influence root phenology. We highlight ways root phenology may respond to climate change differently than aboveground phenology, such as the fact that root phenology may respond differently to warming than aboveground phenology. We suggest that current ecosystem models could be improved by removing the assumption that roots and shoots respond similarly to warming. We also show that ecosystem respiration models may be accurate even if they do not include root phenology. I hope these findings and suggestions aid in future studies of root phenology. As climate change continues to increase temperatures, it is imperative that we understand how plants will play a role in mediating or exacerbating associated changes in atmospheric carbon. A complete understanding of whole-plant phenology will go a long way toward advancing these goals.

References


Appendix A

Supplement to Chapter 5

Figure A1. Relative new root production (new roots produced \( \cdot \text{day}^{-1} \) on that date/maximum new roots produced \( \cdot \text{day}^{-1} \) in that season) in 2016 in graminoids, mixed graminoids and shrubs, and shrubs (Betula nana). Solid gray lines represent graminoid plots, solid black lines represent shrub plots, and dotted black lines represent mixed plots. Error bars indicate ±1 standard error of the mean.

Comparing minirhizotron estimates to destructive harvest measurements:

Measurements of root length \((\text{cm cm}^{-2})\) from minirhizotrons in harvest plots were compared to root length density \((\text{cm cm}^{-3})\) in soil cores taken from harvest plots (Figure S2A). The general pattern was similar, although there were many fewer roots on minirhizotron tubes. This is to be expected, given that roots only grew on the tubes for three years, and roots in this system grow for about five years and may then take years to decompose.
When separated by depth, root length obtained from minirhizotrons was correlated with root biomass obtained from soil cores ($R^2 = 0.89$, $P = 0.057$). This relationship was obtained from a generalized linear mixed model comparing minirhizotron estimates of root length (cm cm$^{-2}$) to estimated living root biomass (g m$^{-2}$) with depth nested within plot as a random variable. When average root length over an entire tube was compared to the sum of root biomass over an entire core, we found no relationship ($R^2 = 0.0088$, $P = 0.66$).

**Figure A2.** Comparing minirhizotron measurements in destructive harvest plots to roots obtained from soil cores in the same plots. Measurements of root length from minirhizotrons (cm cm$^{-2}$) are separated by depth and compared to measurements of root length density (cm cm$^{-3}$) obtained from soil cores.
Laura Radville

Education:
Pennsylvania State University; Ph.D. in Ecology and Ecosystem Science and Management
University of Rhode Island; M.S. in Biological Sciences
College of the Holy Cross; B.A. in Biology and Studio Art

Refereed Publications:

Selected presentations:
Radville, L. Above- and belowground phenology may not be linked. Ecological Society of America annual meeting. Fort Lauderdale, FL. 7-15 Aug. 2016.

Funding awards:
2015: Frank A. Andersen Ecology Travel Award, Ecology Graduate Program, Penn State
2012-2014: Achievement Reward for College Scientists (ARCS) Foundation Scholarship
2012-2013: Pennsylvania State University Distinguished Graduate Fellowship (10,000.00)
2011: Student Travel Award, Applied Ecology Section, Ecological Society of America
2010: Sigma Xi Grant-in-Aid of Research