

Plant diversity, physiology, and function in the face of global change

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ABSTRACT

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One central goal in ecology is to understand how biodiversity, and key organismal traits, interact with ecosystem properties and processes, and ultimately to understand and predict how these interactions will be affected by rapid environmental change. Thus, global change experiments and observational gradients in diversity provide the opportunity to examine and test hypotheses about how organismal traits, multiple dimensions of biodiversity, and ecosystem function will respond to environmental change. In Arctic tundra, increased nitrogen (N) and phosphorus (P) availability accompanying rapid warming is thought to significantly alter plant community composition and ecosystem function. The following four chapters examine hypotheses about the responses of species' traits, multiple dimensions of biodiversity, and ecosystem function to the effects Arctic warming. Chapter 1 examines plant community composition and the capacity for ecosystem function (net ecosystem exchange, ecosystem respiration, and gross primary production) across a gradient of experimental N and P addition expected to more closely approximate warming-induced fertilization, demonstrating declines in plant diversity and an increase in the capacity for ecosystem carbon uptake at the highest level of fertilization. Chapter 2 examines a set of physiological and functional leaf traits across the same N and P gradient in order to evaluate the possible physiological mechanisms underlying community and ecosystem responses, highlighting the effects of increasing nutrient availability for deciduous shrub species. Chapter 3 found that single-dose, long-term nutrient addition (i.e.,

> 20 years) led to significant declines in multiple dimensions of diversity (taxonomic, functional and phylogenetic), and that these effects persist through time, increasing for dimensions that capture organismal traits (functional and phylogenetic). Finally, Chapter 4 examined the relationship between multidimensional diversity and ecosystem function across a natural gradient of diversity, and found that taxonomic diversity and functional diversity were significantly and positively related to whole ecosystem productivity, and, conversely, functional evenness and dispersion were significantly and negatively related to ecosystem productivity. Cumulatively, these four chapters advance our understanding of the connections between communities and ecosystems in a rapidly changing ecosystem.

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For James Martin Prager

INTRODUCTION

As human-altered landscapes now dominate our earth, great emphasis has been placed on understanding how species are impacted by human-induced environmental change, and the consequences of shifts in biological diversity for ecosystem and Earth-system functioning (Cardinale et al. 2012, Naeem et al. 2012). The body of work linking biodiversity (and organismal traits) with ecosystem function has grown exponentially since its emergence just two decades ago, and a great amount of effort has been placed on developing theory and empirical methods to describe the nature of the relationship between biodiversity and ecosystem function (BEF) and the mechanisms (selection effect and niche complementarity) that are thought to underlie these connections (Loreau and de Mazancourt 2013, Tilman et al. 2014). This dissertation combines data generated from long-term experiments and observational gradients in order to better understand and predict the effects of Arctic warming on the relationships between plant diversity and ecosystem function with an attention to the physiological mechanisms that underlie these linkages. Specifically, this dissertation centers on the interactions between warming-related nutrient enrichment, multiple dimensions of biodiversity, functional and physiological leaf traits, and ecosystem CO₂ exchange across low Arctic tundra (Figure 1).

Experimentally, taxonomic and functional-group diversity, principally of producer species, has been shown to exhibit positive, saturating relationships with ecosystem function – typically some measure of productivity – most markedly at low levels of species diversity, often in artificially-assembled grassland systems (Tilman et al. 1997a, Tilman et al. 1997c, Chapin et al. 1998, Reich et al. 2004). Less is known, however, about the causal relationship between diversity and function across naturally assembled systems, or systems undergoing long-term warming or nutrient fertilization, elsewhere on the globe (Bunker et al. 2005, Hooper et al.

2005). In addition, biodiversity is a complex, dynamic and often scale-dependent entity that cannot be readily reduced to a single value or dimension. As human-induced biodiversity losses are escalating, accurately measuring the change in relevant dimensions of biodiversity (i.e. genetic, taxonomic, functional, phylogenetic, structural, etc.) in order to best predict potential ecological consequences is paramount (Purvis and Hector 2000, Naeem et al. 2016b). In the BEF framework, traditional approaches often involve examining species and/or functional group richness, but employing a multidimensional approach may be the most comprehensive strategy for understanding the mechanisms underlying diversity's influence over ecosystem function (Wright et al. 2006, Carroll et al. 2011, Naeem et al. 2016b).

The overarching aim of this dissertation is to understand the connections between plant physiology and multiple dimensions of plant diversity and to explore how these connections are impacted by an indirect effect of global environmental change, nutrient enrichment. In addition, the studies included in this dissertation explore the relationships between organismal traits, multiple dimensions of diversity, and larger scale ecosystem processes, such as ecosystem CO₂ exchange. Each chapter tests hypotheses about how plant traits, species functional, phylogenetic and taxonomic relationships, and ecosystem function are expected to respond to warming-related nutrient enrichment.

Study system

High-latitude ecosystems have experienced rapid warming in recent decades. Mean Arctic surface temperature has increased by 2°C over the past 50 years compared to an increase of approximately 0.72°C in global mean surface temperature (IPCC 2013). Consequently, Arctic tundra ecosystems are predicted to be affected more by warming than any other terrestrial

ecosystem due to a network of positive feedbacks among regional temperature, water vapor, albedo, snow depth, permafrost thaw, and sea ice extent (Chapin et al. 2005, Serreze and Francis 2006, Hinzman et al. 2013).

The effects of Arctic warming include a deepening active (soil) layer and increased soil nutrient mineralization, resulting in the fertilization of a historically nitrogen (N)- and phosphorus (P)-limited landscape (Shaver and Chapin 1986, Chapin 1991). Greater nutrient availability is thought to lead to shifts in plant community composition due to increases in the relative abundance of woody, deciduous shrub species, with consequences for key ecosystem functions such as carbon (C) and nutrient cycling (Rastetter et al. 1991, Hobbie and Chapin 1998, Myers-Smith et al. 2011). In addition, increased nutrient availability is expected to stimulate primary production, enhancing aboveground biomass and ecosystem C gain and belowground productivity and C cycling (Hobbie et al. 2002, Hill and Henry 2011).

The subsequent four chapters are based on data collected near Toolik Lake, Alaska on the North Slope of the Brooks Range between June 2013 and August 2015. Three of the four studies presented here utilized long-term global change experiments at the Arctic Long-Term Research site (ARC LTER) – two of the four chapters use a long-term nitrogen (N) and phosphorus (P) enrichment gradient experiment established in 2006, and one chapter uses data from ARC LTER single-dose N and P addition experiments initiated in 1989, 1996 and 1997. The final chapter uses data from an observational gradient of naturally assembled plant communities, not exposed to any experimental treatment, near Toolik Lake and the ARC LTER.

Thesis structure

Chapter 1 (Published as Prager et al., 2017 in Ecology and Evolution) examines the

impact of increasing N and P availability on plant diversity and ecosystem function.

Specifically, this chapter estimates the amount of N released in association with warming-related permafrost thaw and explores the consequences of a gradient of N and P addition on species richness, abundance-weighted measures of plant diversity and three key measures of ecosystem CO₂ exchange: net ecosystem exchange (NEE), ecosystem respiration and gross primary production. In addition, this chapter compares measured ecosystem CO₂ flux data to a widely used Arctic ecosystem exchange model to investigate the ability to predict the capacity for CO₂ exchange with nutrient addition.

Chapter 2 expands on the findings of Chapter 1, exploring the species-specific physiological responses of Arctic tundra vegetation to increasing nutrient availability. Specifically, Chapter 3 examines how a set of leaf nutrient and physiological characteristics of eight dominant plant species, spanning four plant functional groups, respond to experimental N and P enrichment. This chapter highlights the importance of quantifying and monitoring physiological mechanisms that perhaps underlie changes at the community and/or ecosystem level, aiding in a more comprehensive understanding of the impacts of global change on larger scale processes and properties.

Chapter 3 explores the impact of long-term (i.e., > 20 years), single-dose N and P enrichment on three relevant dimensions of biodiversity (i.e., functional, phylogenetic and taxonomic) in Arctic tundra. While it is generally understood that biodiversity is multidimensional, little attention has been given to integrating multiple dimensions of diversity in assessments of the effects of global change on communities and ecosystems, possibly underestimating the magnitude of the impact of global change on natural systems.

Finally, Chapter 4 examines simultaneous changes in multiple dimensions of plant

diversity (i.e., functional, phylogenetic and taxonomic) and whole ecosystem productivity across Arctic tundra (both NEE and aboveground biomass). As the majority of existing work linking biodiversity and ecosystem function (BEF) is experimental, and far less is understood about the causal relationship between biodiversity and function across naturally assembled systems, this chapter advances the BEF framework to naturally-assembled Arctic ecosystems undergoing rapid environmental change. In addition, this chapter examines multiple dimensions of diversity simultaneously, permitting inferences into possible mechanisms underlying the connections between diversity and function in this system.

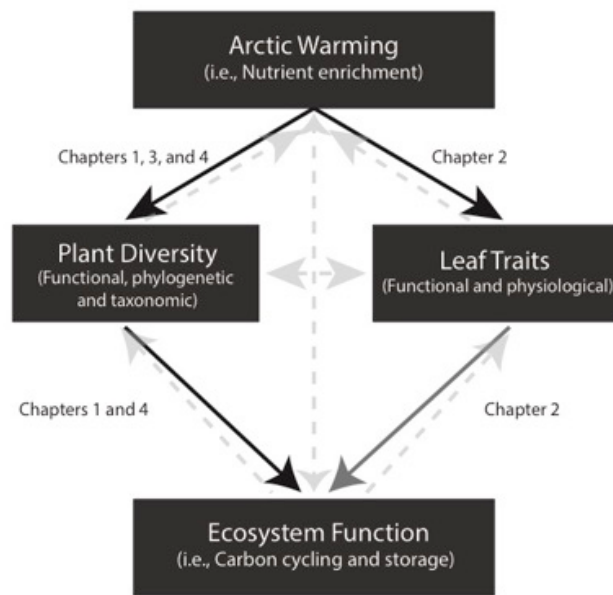


Figure I.1 1

Figure I.1. Conceptual diagram linking the four data chapters presented in this dissertation.

Solid black lines represent measured connections between areas of investigation with arrows representing the hypothesized direction tested and solid grey arrows indicate links that were not explicitly evaluated, but findings from specified chapters have direct implications for those

relationships. Grey dashed lines represent all hypothetical connections between study areas not covered by this dissertation.

Chapter 1 - A gradient of nutrient enrichment reveals non-linear impacts of fertilization on Arctic plant diversity and ecosystem function

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Abstract

Rapid environmental change at high latitudes is predicted to greatly alter the diversity, structure, and function of plant communities, resulting in changes in the pools and fluxes of nutrients. In Arctic tundra, increased nitrogen (N) and phosphorus (P) availability accompanying warming is known to impact plant diversity and ecosystem function; however, to date, most studies examining Arctic nutrient enrichment focus on the impact of relatively large (> 25x estimated naturally occurring N enrichment) doses of nutrients on plant community composition and net primary productivity. To understand the impacts of Arctic nutrient enrichment, we examined plant community composition and the capacity for ecosystem function (net ecosystem exchange, ecosystem respiration, and gross primary production) across a gradient of experimental N and P addition expected to more closely approximate warming-induced fertilization. In addition, we compared our measured ecosystem CO₂ flux data to a widely used Arctic ecosystem exchange model to investigate the ability to predict the capacity for CO₂ exchange with nutrient addition. We observed declines in abundance-weighted plant diversity at low levels of nutrient enrichment, but species richness and the capacity for ecosystem carbon uptake did not change until the highest level of fertilization. When we compared our measured data to the model, we found that the model explained roughly 30-50% of the variance in the observed data, depending on the flux variable, and the relationship weakened at high levels of enrichment. Our results suggest that while a relatively small amount of nutrient enrichment impacts plant diversity, only relatively large levels of fertilization – over an order of magnitude or more than warming-induced rates – significantly alter the capacity for tundra CO₂ exchange. Overall, our findings highlight the value of measuring and modeling the impacts of a nutrient enrichment gradient, as warming-related nutrient availability may impact ecosystems differently than single-level fertilization experiments.

Introduction

High-latitude ecosystems have experienced rapid warming in recent decades. Mean Arctic surface temperature has increased by 2°C over the past 50 years compared to an increase of approximately 0.72°C in global mean surface temperature (IPCC 2013). Arctic tundra is warming rapidly due to a network of positive feedbacks among regional temperature, water vapor, albedo, and associated variations in snow depth, permafrost thaw, and sea ice extent (Chapin et al. 2005, Serreze and Francis 2006, Hinzman et al. 2013). Consequently, Arctic tundra ecosystems are predicted to be affected more by warming than any other terrestrial ecosystem (IPCC 2013).

The effects of Arctic warming are complex and diverse, including a deepening active layer, increased soil nutrient mineralization and subsequent fertilization of a historically nitrogen (N)- and phosphorus (P)-limited landscape (Shaver and Chapin 1986, Chapin 1991). Greater nutrient availability is thought to lead to shifts in plant community composition and physical structure due to increases in the relative abundance of woody, deciduous shrub species, with consequences for key ecosystem functions such as carbon (C) and nutrient cycling (Rastetter et al. 1991, Hobbie and Chapin 1998, Myers-Smith et al. 2011). In addition, increased nutrient availability is expected to stimulate primary production, enhancing aboveground biomass and ecosystem C gain and belowground productivity and C cycling (Hobbie et al. 2002, Hill and Henry 2011), as has been shown by modeling efforts (Jiang et al. 2016). However, recent assessments suggest that, regardless of shifts in aboveground biomass and ecosystem productivity, concurrent increases in organic matter decomposition are weakening the strength of the Arctic CO₂ sink (Hayes et al. 2011), and the region is likely to become a net C source to the atmosphere by 2100 (Abbott et al. 2016). As high-latitude ecosystems contain twice as much C as there is presently in the atmosphere (Zimov et al. 2006, Tarnocai et al. 2009), more than three

times the C in global forest biomass (Houghton 2007), and between a quarter and a third of the globe's total C pools (Carvalhais et al. 2014, Schimel et al. 2015), understanding the ecological consequences of rapid warming and a growing nutrient pool for leaf, community, and ecosystem processes across Arctic tundra ecosystems is paramount.

The majority of nutrient addition experiments – across all ecosystems – aim to examine the extent of nutrient limitation on annual net primary productivity (NPP) (LeBauer and Treseder 2008). To do so, nutrients are often added at levels that far exceed plant demand – at times an order of magnitude greater than predicted deposition or warming-induced increases in nutrient availability (Hobbie et al. 2002). Experimental N and P additions have been used to simulate enrichment in Arctic tundra ecosystems as warming is thought to increase nutrient availability via increases in active layer depths and accelerations in the decomposition of soil organic matter (Hartley et al. 1999, Schimel et al. 2004, Aerts et al. 2006). Such large dose, long-term fertilization experiments (i.e., annual additions of $\geq 10 \text{ g m}^{-2} \text{ yr}^{-1} \text{ N}$ and $\geq 5 \text{ g m}^{-2} \text{ yr}^{-1} \text{ P}$) across varying Arctic tundra types have documented increases in NPP and pronounced shifts in plant community composition and physical structure over time (Shaver and Chapin 1986, Shaver et al. 1998, Boelman et al. 2003, Boelman et al. 2005) often occurring in connection with increases in the abundance of deciduous woody shrub species and decreases in evergreen, grass/sedge, and moss cover (Shaver and Chapin 1986, Shaver et al. 1998).

Shifts in the evenness and dominance of plant species, and declines in plant diversity, are often attributable to shifts in competitive interactions between plant species with increasing nutrient availability (Tilman 1984, 1987) and the competitive displacement of low stature species due light limitation (Goldberg and Miller 1990). One study has shown that high levels of N and P fertilization doubled NPP, but soil C – a much larger pool – decreased substantially, resulting

in a net decrease of ecosystem C storage (Mack et al. 2004). In contrast, examination of more gradual shifts in nutrient availability via long-term warming showed increases in plant biomass and dominance of woody shrub species with no changes in total soil C and N pools, ultimately increasing net ecosystem C storage after 20 years (Sistla et al. 2013). However, it is unclear how much of this response was driven by direct effects of temperature increases vs. indirect effects of warming-related nutrient enrichment (Sistla et al. 2013). In addition, large-scale experimental and observational warming studies have documented increases in deciduous shrub cover that is often indirectly attributed to nutrient enrichment (Elmendorf et al. 2012a, Elmendorf et al. 2012b). While substantial variation in the structure and composition of tundra vegetation exists, previous work has illuminated relatively consistent relationships between productivity and biomass, and canopy leaf area and nutrient use or allocation (Shaver and Chapin 1995, Shaver et al. 1998, Williams and Rastetter 1999). These findings point to the functional convergence of canopies – suggesting similar controls over canopy-level C exchange regardless of any compositional differences in plant communities (Williams and Rastetter 1999, Williams et al. 2001, Shaver et al. 2007, Street et al. 2007) – regardless of the impacts of any variation in resource availability not captured by canopy leaf area.

Monitoring plant community and ecosystem responses across a gradient of fertilization may reveal important dynamics and relationships between plant nutrient availability and use. For example, there may be a point at which plant nutrient availability or uptake outpaces utilization, or non-linear relationships may emerge between nutrient enrichment and ecosystem function (Aber et al. 1998, Bai et al. 2010). Further, experiments that have added a range of N and P levels report shifts in diversity or biomass at all levels of nutrient addition (Tilman 1987, Bowman et al. 2006, Britton and Fisher 2007), suggesting that ecosystem properties or processes

may be impacted by low levels of enrichment (Clark and Tilman 2008). Addressing both the magnitude and variability of nutrient enrichment in a changing world is important if we are to improve our overall understanding of the effects of nutrient availability on plant communities and ecosystem function.

The few incremental nutrient addition experiments that have been conducted in Arctic tundra have found community-level responses to small differences in nutrient enrichment. One study in Northwest Greenland found that ecosystem CO₂ exchange, vegetation cover, and composition were highly sensitive to low rates (i.e., 0.5 g N m⁻² y⁻¹) of N input just 1-2 years after fertilization, suggesting that small increases in N availability have the potential to alter ecosystem structure and function in the high Arctic (Arens et al. 2008). However, subsequent N addition from 1 to 5 g N m⁻² y⁻¹ did not further alter CO₂ exchange or vegetation characteristics, possibly indicating ecosystem N saturation (Arens et al. 2008). In addition, recent leaf-level work in low Arctic Alaska illuminated species-specific decoupling of respiration and photosynthesis and shifts in leaf nutrient content across a nutrient enrichment gradient, with possible consequences for ecosystem carbon balance (Heskel et al. 2012).

In this study, we sought to examine the effects of incremental N and P enrichment on plant community composition and ecosystem function in low Arctic tundra. Specifically, we examined how plant diversity, canopy leaf area, and key components of the capacity for ecosystem C cycling (i.e., net ecosystem exchange (NEE), ecosystem respiration (ER), and gross primary productivity (GPP)) respond to a gradient of experimental N and P enrichment at a low Arctic tundra site in northern Alaska. As we were interested in how the maximum capacity for ecosystem CO₂ exchange was impacted by the magnitude of nutrient addition, and not how nutrient addition impacts CO₂ exchange throughout a season, we focused on measuring

ecosystem processes during the period of peak tundra greenness. In addition, we explored the potential to scale up our findings from the plot to the ecosystem by comparing predictions of CO₂ fluxes derived from a widely-used Arctic ecosystem CO₂ exchange model developed by Shaver et al. (2007) to our measured CO₂ flux data. We also used this model to help partition CO₂ flux responses to nutrient enrichment between various drivers (i.e., leaf area, irradiance or temperature). Overall, we hypothesized that plant diversity (e.g., species richness and abundance-weighted diversity) and ecosystem function (e.g., NEE, GPP, ER) would respond to relatively low levels of nutrient addition.

Methods

Site description and experimental manipulation

All field sampling for this study took place during peak growing season (i.e., the period of peak tundra greenness) across a long-term nitrogen (N) and phosphorus (P) enrichment experiment established in 2006 by G. Shaver and colleagues at the Arctic Long Term Ecological Research (ARC LTER) site, located at Toolik Lake in the northern foothills of the Brooks Range, Alaska (68°38'N and 149°43'W, 760 m a.s.l.). The nutrient addition gradient is located on moist acidic tundra with soils comprised of 30–55 cm of a peaty organic and silty mineral layer, atop continuous permafrost. Each year, following snowmelt but before leaf-out, granular ammonium nitrate and superphosphate is distributed on each 5 × 20 m plot, corresponding to fertilization treatment. Treatment name denotes the amount of fertilizer applied at the beginning of each growing season: “CT”, a control that receives no fertilizer; “F0.5” (0.5 g N m⁻² yr⁻¹ + 0.25 g P m⁻² yr⁻¹); “F1” (1 g N m⁻² yr⁻¹ + 0.5 g P m⁻² yr⁻¹); “F2” (2 g N m⁻² yr⁻¹ + 1 g P m⁻² yr⁻¹); “F5” (5 g N m⁻² yr⁻¹ + 2.5 g P m⁻² yr⁻¹); and “F10” (10 g N m⁻² yr⁻¹ + 5 g P m⁻² yr⁻¹). The nutrient

enrichment plots are replicated in a complete three-block design, resulting in 18 sampled treatment plots, and blocks are positioned roughly 50-100 m apart. The growing season at the ARC LTER site spans 10-12 weeks, beginning in early to mid-June, with an average growing season temperature of 10°C. The period of peak tundra greenness for low Arctic tundra plant communities that are dominated by graminoids and evergreen shrubs is approximately 30-35 days (Sweet et al. 2015). As the growing season in this system is short, we focused on measuring plant community properties and ecosystem function during the period of peak tundra greenness to ensure that we were examining the effects of nutrient addition, and not seasonality, on plant communities and the maximum capacity for ecosystem function.

To compare the magnitude of the experimental nutrient additions to that of naturally occurring fertilization, we calculated a rough estimate of thawing related nutrient enrichment. To do so, we combined data on bulk soil N from Arctic tundra soils (Mack et al. 2011), the change in annual maximum thaw depth from 2000 to 2012 at the Toolik Lake LTER (Shaver and Laundre 2012), ANPP from Arctic tundra (Shaver 2013), and tissue N content (Field and Mooney 1986, Jackson et al. 1997). Assuming steady state of the pre-thawing soil pool, we estimated a mineralization rate constant, which we used to estimate thawing-driven N mineralization (see Appendix S1 for detailed calculation). According to this calculation, naturally occurring enrichment due to thawing permafrost is around 0.3 g N m⁻² yr⁻¹, which falls just below the lowest nutrient enrichment treatment in our study. We suspect that the true thawing-driven nutrient enrichment is likely lower than this (see Appendix S1).

Leaf area index, plant community composition, and plant diversity

To calculate leaf area index (LAI; m² one-sided green leaf m⁻² ground), we used the Normalized Difference Vegetation Index (NDVI). Derived from reflectance data, NDVI captures the relative amount of green vegetation, and thus is an indicator of canopy “greenness” (Rouse et al. 1974). NDVI has proven to be sensitive to differences in aboveground plant structure, biomass and canopy cover in Arctic tundra ecosystems (Vierling et al. 1997, Boelman et al. 2003, Steltzer and Welker 2006, Boelman et al. 2011). We obtained spectral reflectance measurements during peak tundra greenness (July 12-20, 2015) for a subset of at least two of our CO₂ flux locations per plot (N=39) with a field portable double channel spectrometer (UniSpec DC, PP Systems, Amsbury, M.A., USA). The foreoptic was held 1 m above the top of the canopy, with a circular footprint of approximately 0.15 m² and a 40 cm diameter field of view. Three measurements were made within each sampled flux quadrat (roughly 0.75 m in diameter) and averaged to capture spatial heterogeneity. Each vegetation upwelling radiance measurement was immediately followed by a measurement of a 99% reflectance standard from a white Spectralon™ disc (LabSphere, North Sutton, N.H., USA). By dividing the reflected vegetation radiance by the spectralon radiance, we obtained a value for spectral reflectance. NDVI values were calculated from spectral reflectance measurements using Eqn (1), where NIR indicates reflectance at 800 nm [a near-infrared (NIR) wavelength], and R is reflectance at 660 nm [a visible red (R) wavelength]. The NDVI values at each CO₂ flux plot were averaged to obtain a mean value.

$$\text{NDVI} = (\text{NIR} - \text{R}) / (\text{NIR} + \text{R}) \quad (1)$$

Mean NDVI for each flux plot was used to estimate LAI using a model developed by Street et al. (2007) for varying tundra vegetation types, generalized by Shaver et al. (2007) (Eqn 2). This model is commonly employed in studies of Arctic vegetation and carbon fluxes (Lorantý et al.

2011, Street et al. 2012, Shaver et al. 2013, Sweet et al. 2015) and it assumes that differences in NDVI during the period of peak leaf-out (when our study was conducted) are primarily the result of changes in tundra leaf area.

$$\text{LAI} = 0.0026e^{8.0783 \cdot \text{NDVI}} \quad (2)$$

To examine treatment effects on plant diversity, we analyzed percent cover during the period of peak tundra greenness (July 13 – 16, 2012) by using data available through the LTER data portal (<http://ecosystems.mbl.edu>), collected at eight 1-m² quadrats within each 5 x 20 m treatment plot). We used these percent cover data to calculate the number of species in the community, species richness (S), and two common abundance-weighted diversity metrics, the Shannon Index (Eqn 3) and the Simpson Index (Eqn 4), that represent the evenness and dominance of species in a community, where P_i is the fraction of the community made up of species i and S is the species richness of a given community.

$$H = -\sum_{i=1}^S (P_i * \ln P_i) \quad (3)$$

$$D = \frac{1}{\sum_{i=1}^S P_i^2} \quad (4)$$

All measures of diversity were calculated using the *vegan* package (Okasen et al. 2015) in R v. 3.2.1 (R Core Team 2015). As plant percent cover data were taken at eight subplots within the control, F0.5, F2, F5 and F10 treatment plots, they are an accurate, thorough representation of plant communities across the experimental plots. Given the short growing season and the large abundance of perennial and evergreen species with conservative growth strategies in this system (Bliss and Petersen 1992) plant communities likely shift slowly, rather than abruptly, in response to environmental change and resource availability (Camill and Clark 2000, Dormann and Woodin 2002). In addition, previous work in this region has shown that plant percent cover in

this system is unlikely to change over short (e.g., < 5 years) temporal scales (Jorgenson et al. 2015).

Measured CO₂ flux measurements and calculations

During the period of peak tundra greenness (July 12-16, 2015), changes in CO₂ concentration, water vapor, photosynthetically active radiation (PAR) and air temperature were measured using a Li-Cor 6400XT infrared gas analyzer (IRGA; Li-Cor, Lincoln, Nebraska, USA) operated in closed-system mode. The IRGA was affixed to a transparent, cylindrical, portable polycarbonate chamber (r= 0.36 m; h=0.61 m), with internal fans to ensure adequate mixing of air and steady chamber temperatures, atop a separate base (r=0.37 m; h=0.15 m) fitted with a plastic skirt, sealed to the ground with two heavy chains. Because the range of LAI values across all plots was relatively small (Fig. 4a), the same chamber was used for all gas exchange measurements. At each sampling location, we conducted flux measurements to permit calculation of both net ecosystem exchange (NEE) and ecosystem respiration (ER). Each measurement cycle began by lowering the chamber onto the base and sealing it. Once a consistent rate of CO₂ exchange was achieved, we began logging a 40-second flux measurement – following a method similar to the International Tundra Experiment (ITEX) and that of Shaver et al. (1998), Shaver et al. (2007) and (Shaver et al. 2013) – in the light (for calculation of NEE) at a sampling frequency of 0.5 Hz. Once we completed a flux measurement in the light, the flux chamber was covered with an opaque black cloth and allowed to acclimate for 15-30 seconds before logging a 40-second flux measurement in the dark (for calculation of ER). This cycle was repeated five times, yielding five flux measurements in the light and five in the dark at each sampling location. The temperature in the chamber did not exceed 25.2 °C during any

measurement, and conditions for each repeated measure were stable. For each sampling location within each treatment plot, we averaged the five fluxes made in the light and the dark respectively and we calculated three relevant flux metrics: NEE, ER, and gross primary production (GPP). Measurements from the three sampling locations were averaged to obtain a mean value for each treatment plot, resulting in three observations per treatment (one mean value per treatment per block), and outliers were removed prior to averaging.

To calculate NEE ($\mu\text{mol m}^{-2} \text{s}^{-1}$), we used Eqn 5 to quantify the continuous exchange of CO_2 between the atmosphere, vegetation and soil in the light. In Eqn 5, ρ is the air density (mol air m^{-3}), defined as $\frac{P}{RT}$, where P is the average pressure (Pa), R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ air K}^{-1}$), and T (K) is the mean temperature. V is the chamber volume (m^3), dC/dt is the slope of the chamber CO_2 concentration against time ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air s}^{-1}$), and A is the surface area of the ground (m^2) within the chamber. Negative NEE values indicate fluxes from the atmosphere to the ecosystem and positive values indicate fluxes to the atmosphere from the ecosystem.

$$\text{NEE} = (\rho * V * (dC/dt) / A) \quad (5)$$

$$\text{GPP} = \text{ER} - \text{NEE} \quad (6)$$

In addition, we calculated ER using Eqn 5 for all flux measurements taken in the dark. We then calculated gross primary production (GPP) as the difference between ER and NEE (Eqn 6).

Modeled CO_2 fluxes

To compare our flux measurements to those predicted at a system-level scale we modeled net ecosystem exchange (NEE_M) using the model developed initially by Shaver et al. (2007)

(Eqn 7 through 9) and further modified by Shaver et al. (2013) which requires input of three variables: LAI, PAR, and air temperature (T). Predicting CO₂ fluxes using only LAI, PAR, and air T, has been shown to produce a reasonable estimation of Arctic tundra CO₂ exchange (Shaver et al. 2007, Street et al. 2007, Rastetter et al. 2010). While the model is often viewed as a bulk NEE model, accurate representations of ER and GPP are critical to determining realistic estimates of NEE. In addition, previous work has shown that robust estimates of NEE, particularly at the landscape scale, require an accurate and mechanistic understanding of both ER and GPP (Lorantý et al. 2011).

PAR and T data were obtained from the Li-Cor 6400XT used for CO₂ flux measurements. PAR and T values were calculated for each of the five measurements made in the light and then averaged to obtain mean values. NEE_M (μmol CO₂ m⁻² s⁻¹) was calculated using Eqn (7) as the difference between modeled ER (ER_M) and GPP (GPP_M) where negative values of NEE_M represent net CO₂ uptake by the ecosystem. While variations on the model exist, we used model parameter values estimated on low Arctic (the bioclimatic region our study was conducted in that lies between the sub-Arctic and high Arctic) datasets that encompass a variety of low Arctic tundra vegetation types (Shaver et al. 2013).

$$NEE_M = ER_M - GPP_M \quad (7)$$

ER_M was calculated using Eqn (8), using parameter values for β , R_0 , and R_X as determined by Shaver et al. (2013). Here, R_0 (1.177 μmol CO₂ m⁻² leaf s⁻¹) is the basal respiration rate, accounting for both autotrophic and heterotrophic respiration, β (0.046 °C⁻¹) is an empirically fit parameter, and T is air temperature (°C). The additional source of respiration in Eqn (8), R_X (0.803 μmol CO₂ m⁻² ground s⁻¹), corresponds to respiration at deeper soil horizons, is independent of LAI and fluctuations in air T, and is included in the model as it

enhances accuracy, model fit, and prevents ER from dropping to zero when there is no canopy leaf area (Shaver et al. 2013).

$$ER_M = (R_0 * e^{\beta * airT * LAI}) + R_x \quad (8)$$

$$GPP_M = (P_{maxL}/k) * \ln((P_{maxL} + E_0 * I) / (P_{maxL} + E_0 * I * e^{(-k * LAI)})) \quad (9)$$

Modeled gross primary productivity (GPP_M) was calculated using Eqn (9) and parameter values for P_{maxL} , k , and E_0 from Shaver et al. (2013), where P_{maxL} ($14.747 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$) is the light-saturated photosynthetic rate per unit leaf area, k ($0.5 \text{ m}^2 \text{ ground m}^{-2} \text{ leaf}$) is a Beer's law extinction coefficient, and E_0 ($0.041 \mu\text{mol CO}_2 \text{ fixed } \mu\text{mol}^{-1} \text{ photons absorbed}$) is the initial slope of the light response curve. Incoming solar irradiance (I) is the top-of-the-canopy photosynthetic photon flux density ($\mu\text{mol photons absorbed m}^{-2} \text{ ground s}^{-1}$). Irradiance is assumed to be the same per leaf area as per ground area at a given layer in the canopy. I was calculated from PAR data recorded by an upward-looking sensor logged by the LiCor 6400XT IRGA; we calculated an average PAR value for each flux location.

Statistical analyses

We used a series of mixed effects models for each of our response variables (e.g., plant diversity and ecosystem function) with treatment as a fixed effect and block as a random effect. Block did not have a significant effect in any of our preliminary analyses. Therefore, to determine the influence of nutrient enrichment on measured and modelled CO_2 fluxes, plant diversity, relative cover of plant functional groups and LAI, we used one-way analyses of variance (ANOVA) followed by Tukey Honest Significance Difference post-hoc tests when ANOVA results were significant ($N=3$ for each treatment level). Linear models were used to compare measured and modeled flux metrics, and we characterized the strength of the

relationship between measured and predicted ecosystem CO₂ flux metrics (i.e., NEE, ER, GPP) using the coefficient of determination (R^2) and root mean squared error (RMSE). In addition, we compared a subset of our measured flux data for which we had LAI values and our modeled fluxes between nutrient treatments using a two-way ANOVA. For all analyses, P -values < 0.05 were considered statistically significant. All analyses were completed in R v. 3.2.1 (R CoreTeam 2015) using the ggplot2 (Wickham 2009), lme4 (Bates et al. 2015), lsmeans (Lenth 2016) and vegan (Okasen et al. 2015) packages.

Results

Leaf area index, plant fractional cover, and plant diversity

We detected a statistically significant difference in leaf area index (LAI) with nutrient addition. LAI was significantly greater in the highest nutrient addition treatment (F10) than in all other treatment levels, except for F5 (Fig. 1). Mean LAI hovered around 1 for the CT, F0.5, F1, and F2 treatments, and mean LAI was 1.19 ($SE = 0.04$) and 1.44 ($SE = 0.09$) at F5 and F10 respectively (Fig. 1). When examining plant community composition and diversity, we found strikingly divergent trends in plant species richness, the number of species in a community (S), and two abundance-weighted measures of plant diversity, the Shannon (H) and Simpson (D) indices, in response to nutrient addition. Species richness did not decrease significantly with nutrient addition until the highest level of enrichment (Fig. 2a), when mean S dropped to 8.5 ($SE = 0.31$) compared to 10.4 ($SE = 0.20$) in control plots. However, when abundance-weighted measures of plant diversity were considered, control plots had 69% and 76% higher H and D index values, respectively, than the lowest nutrient addition treatment (F0.5) which had 39% - 59% higher H values and 41% - 64% higher D values than all other treatment levels (Fig. 2b,c).

In order to further examine shifts in plant communities with nutrient addition, we decomposed our diversity measures and explicitly examined changes in the percent cover of four plant functional groups: deciduous shrubs (e.g., *Betula nana*, *Salix pulchra*, *Vaccinium uliginosum*), evergreen shrubs (e.g., *Empetrum nigrum*, *Vaccinium vitis-idea*), forbs (e.g., *Rubus chamaemorus*, *Polygonum bistorta*), and graminoid species (e.g., *Eriophorum vaginatum*, *Carex bigelowii*). We found statistically significant effects of nutrient addition on deciduous shrub, evergreen shrub and forb cover. The relative abundance of deciduous shrubs was significantly higher at F10 than in the control, and the percent cover of the dominant deciduous shrub species, *B. nana*, was higher in the F10 treatment than in CT, F0.5 and F2 treatment plots. In addition, the relative abundance of forb species was significantly higher at F10 than in control plots and at F0.5. Finally, evergreen shrub cover decreased with nutrient enrichment and was significantly lower at F10 than at F0.5 and F2, and tended toward being significantly lower at F10 than at CT and F5 (both $P < 0.1$). We did not detect statistically significant differences between treatments for graminoid (grass/sedge) cover.

Measured ecosystem CO₂ fluxes

Environmental conditions were relatively stable throughout the sampling period (see Figure S1 in Supporting Information), and there were no statistically significant differences in PAR or T across sampling dates or between nutrient addition treatments (see Figure S2 in Supporting Information). Across all fluxes and treatment plots, measured NEE ranged from -9.12 to -3.61 (M = -5.62, SE = 0.20), ER from 3.73 to 8.69 (M = 5.14, SE = 0.21), and GPP from 8.52 to 16.41 (M = 10.94, SE = 0.38), all $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ ground s}^{-1}$. There were statistically significant differences in GPP ($P < 0.001$), NEE ($P < 0.01$) and ER ($P < 0.05$) across nutrient

addition treatments. NEE values were significantly larger (NEE was more negative indicating larger fluxes to the ecosystem) in the highest nutrient addition treatment (F10) when compared to all other treatments (Fig. 3a). In addition, GPP and ER were higher at F10 than at all other treatments (Fig. 3b,c).

Measured-modeled CO₂ flux comparison

We calculated modeled NEE, GPP, and ER using a model that required the input of three measured variables: LAI, PAR, and T. LAI ranged from 0.58 to 1.63 ($M = 1.11$, $SE = 0.04$) m² leaf m⁻² ground, PAR ranged from 909-1779 ($M = 1406$, $SE = 37.07$) $\mu\text{mol photons m}^{-2}$ ground s⁻¹, and T from 16.58 to 25.17 ($M = 21.94$, $SE = 0.34$) °C (Fig. 4a-c). For modeled values, NEE_M ranged from -11.20 to -3.41 ($M = -7.09$, $SE = 0.30$), ER_M from 2.47 to 6.28 ($M = 4.38$, $SE = 0.14$), and GPP_M from 5.88 to 16.28 ($M = 11.47$, $SE = 0.42$), all $\mu\text{mol CO}_2 \text{ m}^{-2}$ ground s⁻¹. We found similar trends for modeled NEE and GPP as those observed for measured fluxes. NEE and GPP were significantly greater (more negative in the case of NEE) at F10 than all other treatments except for F5 (Fig. 5a,c). We did not find any significant differences in modeled ER across nutrient addition treatments (Fig. 5b).

When we compared our measured flux data to the model developed by Shaver et al. (2007), we found that the model explained 50.9% of the variance in NEE in our dataset, and the root mean square error (RMSE) for measured versus modeled NEE was 1.29 $\mu\text{mol CO}_2 \text{ m}^{-2}$ ground s⁻¹ (Fig. 6a). For GPP, the regression explained 52.4% of the variance and the RMSE was 1.76 $\mu\text{mol CO}_2 \text{ m}^{-2}$ ground s⁻¹ (Fig. 6b). The model explained less of the variance for ER (25.9%) with a RMSE of 1.71 $\mu\text{mol CO}_2 \text{ m}^{-2}$ ground s⁻¹ (Fig. 6c). To assess the role of LAI in our modeled flux calculations, as opposed to temperature or PAR, we re-calculated our

modeled fluxes using randomized LAI values across our dataset, and we found that the model explained less than 8.5% of the variance for all flux variables (i.e., NEE, ER, GPP), with no significant slopes (all $p > 0.05$). The relationship between modeled and measured data appeared to weaken at the highest level of nutrient addition. To examine potential differences between measured and modeled fluxes at the highest nutrient addition treatment, we compared the subset of our measured data for which we had LAI data (Fig. 5a-c) to the modeled CO₂ flux data by treatment level. We found significant differences between measured ER and modeled ER with nutrient addition, and measured ER was significantly higher than modeled ER at the F10 treatment (Fig. 5b). We did not detect statistically significant differences between measured and modeled NEE or GPP with nutrient addition.

Discussion

The goals of this study were to assess how incremental nutrient additions ranging from small to large doses impacted Arctic tundra (1) plant community properties; and, (2) key components of the capacity for ecosystem carbon cycling during the period of peak tundra greenness. Warming-induced nutrient enrichment is presumably a gradual process, and understanding the responses of plant communities and ecosystem function to relatively low levels of nutrient fertilization is a crucial step in predicting ecological responses to global change. We found that a gradient of nutrient enrichment revealed non-linear responses of plant communities and the capacity for ecosystem CO₂ exchange to nutrient manipulations at a low Arctic site, largely deviating from our initial hypotheses. This study advances our understanding of the responses of Arctic plant communities and the capacity for ecosystem function to scenarios of gradual nutrient enrichment that are likely to be more indicative of warming-

induced shifts in nutrient availability than additions of large amounts of N and P that were designed to illustrate and understand Arctic tundra nutrient limitation.

Plant diversity declines with small increases in nutrient availability

Consistent with our hypotheses, we found that plant diversity indices that account for species evenness and dominance declined with just a small amount of nutrient addition (Fig. 2b,c) – the level that is most comparable to our estimate of thawing-induced enrichment. However, nutrient enrichment did not affect species richness (S) until high levels of addition (Fig. 2a). Nutrient-limited ecosystems are often characterized by plant communities with species that differ strongly in their ability to respond to alterations in resource availability; thus, shifts in plant diversity or in species-specific, leaf-level physiology may precede any changes in ecosystem processes and properties (Aber et al. 1998). Long-term (i.e., >20 years) Arctic tundra enrichment experiments with additions comparable to the highest (F10) treatment in this study have documented declines in species richness and shifts in species dominance and evenness with nutrient enrichment (Gough et al. 2002, Gough and Hobbie 2003), as have studies in other biomes (Zavaleta et al. 2003, Suding et al. 2005). However, our findings indicate that after six years of enrichment, plant diversity measures that capture relative abundance are nearly as sensitive to low levels of nutrient fertilization as they are to high levels.

There are a variety of ecological mechanisms that might explain shifts in abundance-weighted plant diversity, but not species richness, with low to moderate levels of nutrient addition. Lower levels of nutrient enrichment of a N- and P-limited system may reduce niche differentiation or complementarity that would otherwise promote species coexistence (Harpole et al. 2011), leading to increases in the relative abundance of species that outcompete neighbors

with lower growth rates (Hautier et al. 2009). We found that large increases in nutrient availability (i.e., F10) ultimately favored deciduous shrub and forb species and led to declines in evergreen shrub cover. These findings are in keeping with previous work in this system that found that after six years of N and P fertilization comparable to the F10 treatment in our study, increases in LAI of the dominant deciduous shrub species, *Betula nana*, and the formation of a dense canopy resulted in light limitation of other shrub species and plant functional types (Bret-Harte et al. 2001). In addition, previous work across our study gradient found that *B. nana* foliar N increased at high levels of fertilization (e.g., F10), but not at low levels, and found no effect of fertilization on foliar N of the dominant graminoid species, *Eriophorum vaginatum* (Heskel et al. 2012). Here, we found that LAI increased with nutrient addition (Fig. 1), as species evenness declined, ultimately resulting in changes in ecosystem CO₂ exchange at high levels of nutrient addition, likely due to the competitive advantage of deciduous shrub species.

High levels of nutrient enrichment impact ecosystem CO₂ exchange

Although low levels of nutrient addition led to declines in plant diversity via shifts in species evenness and dominance, the capacity for ecosystem CO₂ exchange was not impacted until high levels of addition. We found that nine years of nutrient enrichment had significant effects on NEE, ER and GPP at the highest level of addition when NEE became significantly more negative (greater fluxes to the ecosystem) because increasing GPP overcame increasing ER (Fig. 4a-c). The documented responses of plant communities and ecosystem functioning to gradual addition may be due to a variety of mechanisms; however, we focus on three primary explanations: 1) the ecosystem-level consequences of plant adaptations and responses to chronic

nutrient limitation, 2) the role of microbial activity in mediating ecosystem function, and 3) abiotic nutrient sinks and/or losses.

First, as the availability and subsequent uptake of limiting resources is predicted to stimulate primary productivity, we expected to see an immediate increase in GPP and NEE after nine years of low or moderate nutrient enrichment; however, plant communities across chronically nutrient limited landscapes may exhibit lower overall maximum potential growth rates or may allocate resources to belowground structures in order to maximize nutrient uptake and retention (Grime 1977, Chapin 1980, Chapin et al. 1986, Chapin 1991). Thus, increasing nutrient availability may not be diverted to the production of photosynthetic biomass, inducing shifts in below versus aboveground allocation with functional consequences for ecosystem CO₂ exchange, until high levels of fertilization.

Second, nitrogen (N) and phosphorus (P) mineralization in Arctic soils is thought to be low during the growing season, linked to the immobilization of nutrients by microorganisms, contrasted with a high release rate during the winter (Giblin et al. 1991, Nadelhoffer et al. 1991). These findings point to competition between plants and microbes during the growing season that might explain the lag in GPP until a high level of enrichment is reached, possibly driving the decoupling of plant community and ecosystem responses to multi-level nutrient enrichment. While Arctic tundra plant communities are known to be nutrient-limited, tundra microbial communities are also nutrient-limited, as is evidenced by the stimulation of microbial N-immobilization and enhancement of microbial activity with nutrient enrichment (Mack et al. 2004, Lavoie et al. 2011). As a result, nutrient enrichment effects on ecosystem-level process, and specifically the stimulation of ecosystem CO₂ exchange, may not be seen until high levels of fertilization when nutrient availability outpaces microbial utilization.

Finally, there are two abiotic mechanisms that may be responsible for our documented ecosystem-level responses to a gradient of nutrient enrichment: abiotic sinks and leaching. First, abiotic sinks via the adsorption and precipitation of P might initially compete with plants and microorganisms for increasing P availability (Olander and Vitousek 2004), ultimately resulting in a P sink that is not saturated until high levels of experimental P addition (i.e., the F10 treatment in this study). While strong abiotic P sinks are well known, abiotic sinks for N are less well understood, but possibly play a significant role. In addition, leaching of dissolved organic N and nitrate and denitrification may be important loss pathways in this system (Giblin et al. 1991, Mack et al. 2004). Abiotic mechanisms may have dampened the effects of lower levels of nutrient addition, but it is not clear how they might help explain the contrasting responses of plant communities to low levels of addition and ecosystem responses to high levels of enrichment.

Modeled CO₂ fluxes estimate ecosystem responses to low-to-moderate levels of fertilization

As the Arctic continues to warm, our ability to accurately measure, monitor and predict C cycling across large spatial and temporal scales is paramount. This task is challenging as Arctic tundra landscapes are complex and heterogeneous, and are often dominated by varying plant functional groups, with important effects on key components of C cycling (Chapin et al. 2006). However, previous research has shown that canopy C exchange across a wide range of Arctic ecosystems is controlled by the same factors despite pronounced differences in plant community composition, providing evidence of functional convergence (Williams and Rastetter 1999, Williams et al. 2001, Street et al. 2007). As such, modeling efforts have assumed that, regardless of plant diversity or community structure, canopy C exchange can be predicted from leaf area,

light and temperature alone (Shaver et al. 2007). Less is known, however, about how canopy C exchange is impacted by increasing nutrient availability.

When comparing our measured fluxes with fluxes calculated from an Arctic ecosystem exchange model by Shaver et al. (2007), we found that the model explained less of the variance than found in previous studies (e.g. Shaver et al. 2013), though it still explained roughly half of the variance for both NEE (51%) and GPP (52%), and 23% of the variance in ER (Fig. 6a-c). The amount of variance explained dropped to < 8.5% for all three flux variables when we randomized LAI values in our dataset, suggesting that the impact of nutrient addition on LAI, and not temperature or irradiance, is the principle driver of the variation we can account for with this model. However, given the relatively low amount of variance explained for ER, nutrient enrichment appears to have an effect on ER that cannot be explained by canopy leaf area. This effect is perhaps driven by the response of microbial communities to fertilization that is not captured by the parameter in the Shaver et al. (2007) model that represents microbial respiration from deeper soil horizons (R_x), though it is surprising that this is not significant until the highest nutrient addition treatment (Fig. 5b). As previous work in this system has shown that nutrient enrichment stimulates the decomposition of C pools in deeper soil horizons (Mack et al. 2004), incorporating variable R_x values into the model may help account for this discrepancy.

The discrepancy between our measured and modelled data under high nutrient fertilization may also be explained in part by the effects of background reflectance (e.g., the effects of soil or non-foliar vegetation reflectance) on the relationship between NDVI and LAI (Rocha and Shaver 2011). The potential impact of background reflectance, coupled with our observed shifts in community composition and possibly canopy architecture, suggest that NDVI derived LAI may not be an appropriate leaf area estimate for structurally diverse canopies. In

addition, Shaver et al. (2007, 2013) suggest that the success of the model in predicting NEE using just LAI or whole canopy N content is due to a high degree of convergence in canopy structure and function, and our results suggest that high levels of nutrient addition may alter this relationship.

Overall, our data comparison demonstrates that the Shaver et al. (2007) model estimates NEE and GPP relatively well even when plant communities are subjected to resource manipulations (Shaver et al. 2013). However, the diminished ability of the model to accurately estimate ER (Fig. 5b), particularly at high levels of nutrient addition, suggests that further work is needed to understand how to model ecosystem responses to nutrient enrichment. As Arctic systems continue to warm rapidly, accurate estimates of ecosystem CO₂ exchange will be a crucial component of understanding and predicting responses and feedbacks to global change, and our findings suggest that increasing nutrient availability may impact our ability to rely on current model parameterizations.

Implications

To date, results from long-term experiments examining the impacts of large annual doses of nutrients in Arctic tundra have documented significant shifts in plant community composition and dominance, aboveground biomass, and ecosystem function. However, this level of fertilization may be an unrealistic outcome of warming-induced nutrient enrichment in the Arctic. Our study is one of the first to examine how Arctic plant communities and the capacity for ecosystem function (e.g., CO₂ exchange) during the period of peak tundra greenness respond to a gradient of enrichment. We demonstrate that, despite reorganization of plant communities with low levels of addition, significant alteration of ecosystem CO₂ exchange only occurs at the

highest level of nutrient enrichment, suggesting a shift in the capacity for ecosystem C gain only at high levels of fertilization that likely exceed warming-induced enrichment. In addition, we show that examining a gradient of nutrient addition may help identify thresholds past which models intended to upscale estimates of ecosystem function may decline in accuracy – improving our ability to model and comprehend the impacts of global-change. In addition, our results point to the need for further work examining the role of the magnitude of nutrient enrichment on below and aboveground plant community properties and ecosystem processes and any temporal variation in these patterns and relationships.

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Author's Contributions

CMP and KLG conceived the ideas and designed methodology; CMP, HEG, and KLG collected the data; CMP analyzed the data; all authors contributed to the interpretation of analyses; CMP led the writing of the manuscript; all authors contributed critically to the drafts and gave final approval for publication.

Figures

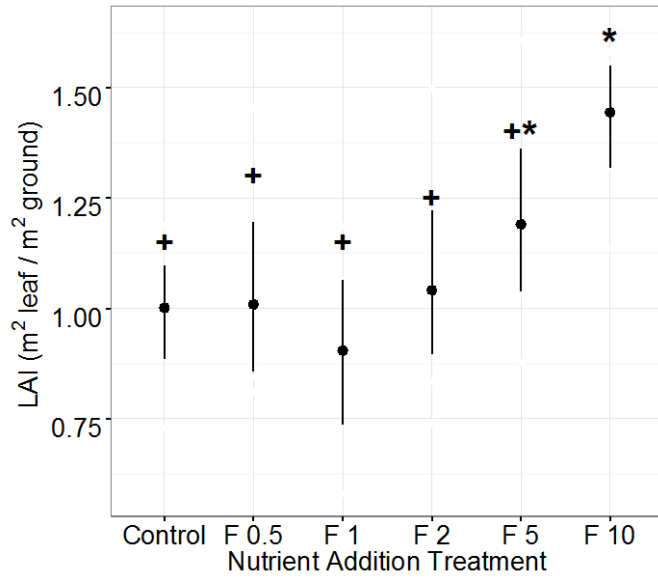


Figure 1.1 1 Leaf area index (LAI) across nutrient addition treatments. Points are mean LAI values (N=3) and error bars represent the standard error (SE) of the mean. LAI increased with nutrient addition; and, LAI was significantly higher at the highest treatment (F10) compared to all other treatments (except for F5) and the control (CT), indicated by non-overlapping symbols.

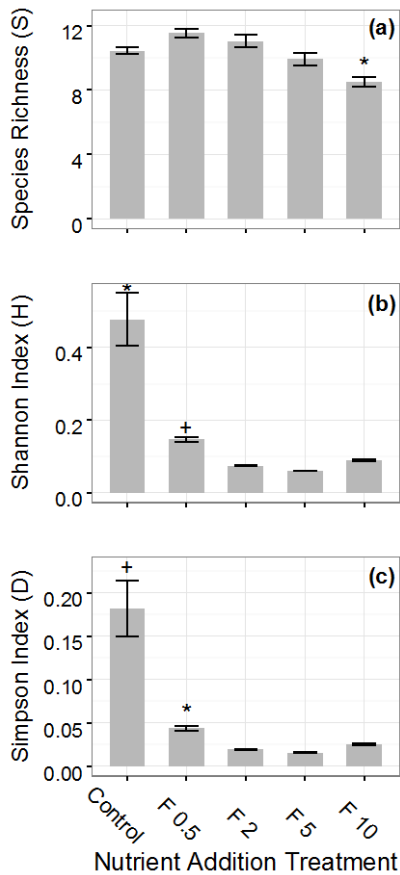


Figure 1.2 1 Plant diversity by nutrient addition treatment. Species richness (S) is the number of vascular plant species in a community and the Shannon (H) and Simpson (D) values are unitless index values representing relative abundances of species in a community. Statistically significant differences are represented by non-overlapping symbols (N=3). Error bars represent the standard error (SE) of the mean. (a) S was significantly lower at the highest nutrient addition treatment (F10) than all other treatments and the control. There were no significant differences between the remaining treatments or between the control and addition treatments. (b, c) H and D, respectively, decreased from the control treatment to the first addition treatment (F0.5) and again between F0.5 and all other addition treatments (F2, F5 and F10).

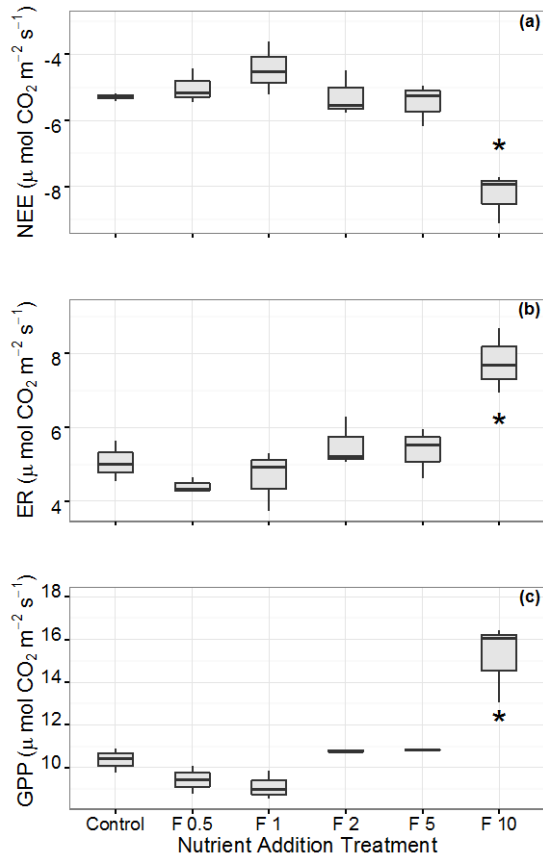


Figure 1.3 1 Boxplots depicting treatment differences between three measured flux variables: net ecosystem exchange (NEE), ecosystem respiration (ER), and gross primary productivity (GPP). Asterisks denote significant differences between means (N=3) at the highest nutrient addition treatment (F10) for all three CO₂ exchange metrics.

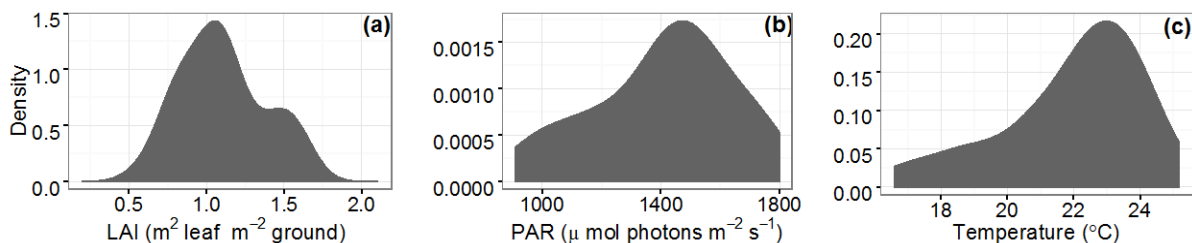


Figure 1.4 1 Frequency distributions of environmental data and leaf area index (LAI) collected during CO₂ exchange measurements for the calculation of predicted CO₂ fluxes using a widely employed Arctic ecosystem exchange model by Shaver et al. (2007). (a) LAI values ranged from 0.58 to 1.63 ($M = 1.11$, $SE = 0.04$) m² leaf m⁻² ground. (b) Photosynthetically active radiation (PAR) ranged from 909-1779 ($M = 1406$, $SE = 37.07$) μmol photons m⁻² s⁻¹. (c) Air temperature across all sampling locations ranged from 16.58 to 25.17 ($M = 21.94$, $SE = 0.34$) °C.

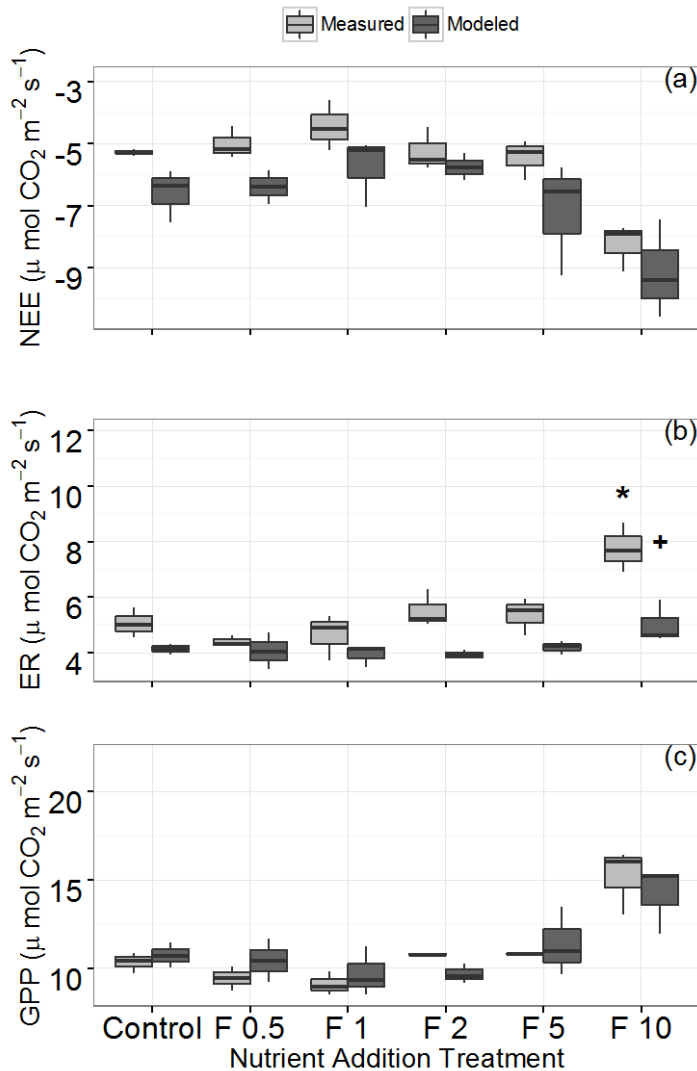


Figure 1.5 1 Boxplots showing a comparison of the subset of measured CO₂ flux data (the subset for which we have leaf area index values) and modelled data across nutrient treatments (N=3 for both modeled and measured data at each treatment level). Statistically significant differences between measured and modeled data within a treatment are indicated by non-overlapping symbols. Measured ER values were significantly higher than modeled values in the F10 treatment. There were no statistically significant differences between measured and modeled NEE or GPP by treatment.

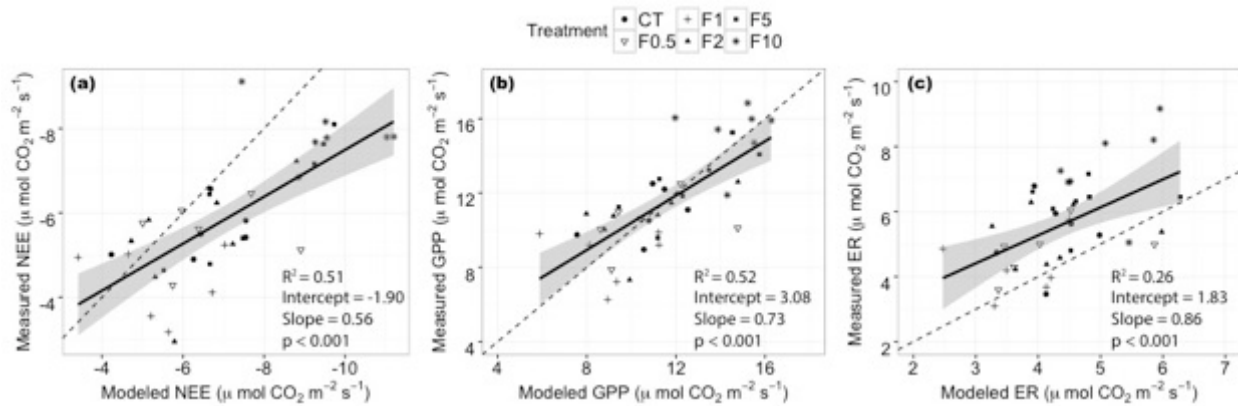


Figure 1.6 1 Relationships between measured and modeled CO₂ flux variables. Predicted fluxes were calculated using a widely employed Arctic CO₂ exchange model by Shaver et al. (2007). All plots include a solid linear regression line, a shaded 95% confidence interval of the regression line, and a dashed one to one line. (a) The model explained 51% of the variance in net ecosystem exchange (NEE) (b) 52% of the variance in gross primary production (GPP); and (c) it explained 26% of the variance in ecosystem respiration (ER).

Supporting Information

Appendix S1. Code used to calculate an estimate of naturally occurring enrichment based on existing, published data on bulk soil N from Arctic tundra soils, the change in annual maximum thaw depth across 12 years at the Toolik Lake LTER, ANPP from Arctic tundra, and tissue N content estimates. In this calculation we assume steady state of the pre-thawing soil pool, and we estimated a mineralization rate constant, which we used to calculate thawing-driven N mineralization. We estimate naturally occurring enrichment due to thawing permafrost at around $0.3 \text{ g N m}^{-2} \text{ yr}^{-1}$, and we note that true thawing-driven nutrient enrichment is likely lower than this estimate.

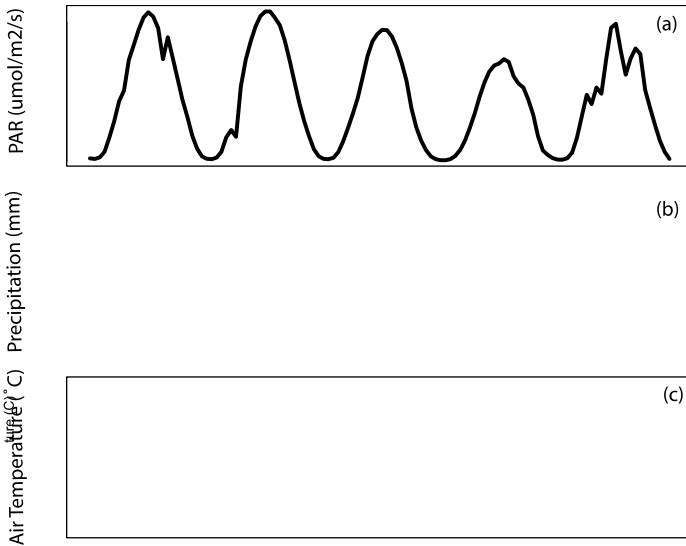


Figure S1.1 1

Figure S1.1 Environmental conditions at the Toolik Field Station meteorological tower during our sampling period (July 12-16, 2015). There were no cloudy days during the sampling period (a), no precipitation events (b), and air temperature was relatively stable, with a slight increase in maximum temperature on the final day of sampling (c).

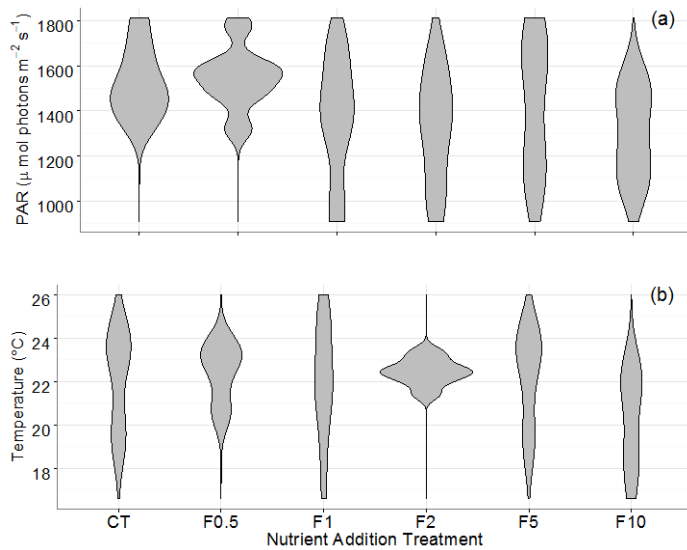


Figure S1.2 1

Figure S1.2 Distribution of light (PAR) and temperature data by nutrient addition treatment.

There were no significant differences in PAR (a) or in temperature (b) between treatments.

Corrigendum to “A gradient of nutrient enrichment reveals nonlinear impacts of fertilization on Arctic plant diversity and ecosystem function”

In a recent paper (Prager et al. 2017), we presented results for abundance-weighted plant diversity measures generated by a plant community composition data file that miscoded plot ID as species abundance. We re-calculated the Shannon and Simpson indices and find that they now follow a similar non-linear trend as species richness and ecosystem function. These changes are reflected in an updated Figure 2 (a-c).

The abstract should reflect this change as “Our results suggest that only relatively large levels of fertilization ... significantly alter the capacity for plant diversity and CO₂ exchange in the

tundra.” In the discussion, the plant diversity sub-heading should now read “*Plant diversity declines with increasing nutrient availability*” followed by “We found that nutrient enrichment did not affect species richness (*S*) or abundance-weighted diversity indices until high levels of addition (Fig. 2a-c).”

We also provide additional information about the plant composition data used for our plant diversity calculations below (Gough 2015).

We thank Laura Gough for uncovering our error, and we also acknowledge an additional funding source, NSF grant DEB-1026843 awarded to G. Shaver and colleagues at the Arctic Long Term Ecological Research (ARC LTER) site.

Authorship

CMP corrected the article, all authors approved the correction.

Citations:

Gough, L. 2015. Relative percent cover of plant species in LTER moist acidic tundra experimental plots and in new experimental plots established in 2006.

<https://doi:10.6073/pasta/eef3bc16b03ca2d9730da9c27c1feb07>

Prager CM, Naeem S, Boelman NT, et al. 2017. A gradient of nutrient enrichment reveals nonlinear impacts of fertilization on Arctic plant diversity and ecosystem function. *Ecol Evol.* 7:2449–2460. <https://doi.org/10.1002/ece3.2863>

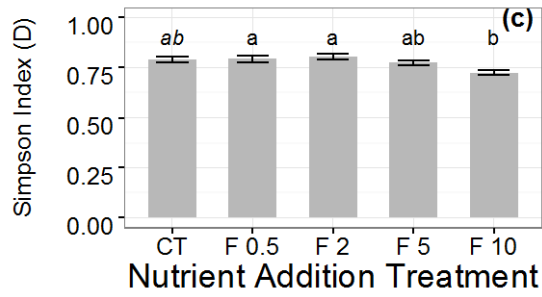
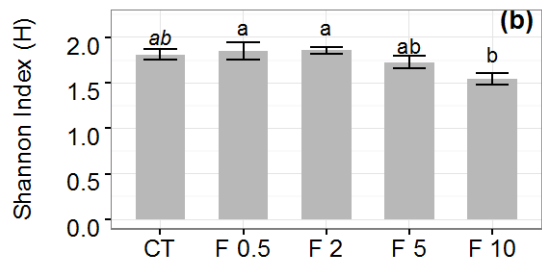
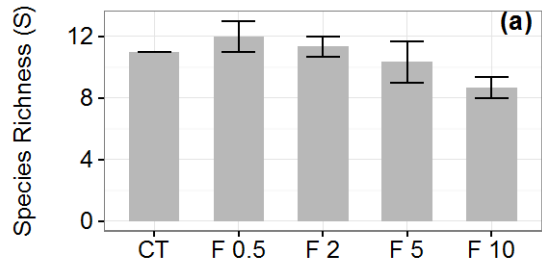


Figure C1.2 1 Plant diversity by nutrient addition treatment. Species richness (*S*) is the number of vascular plant species in a community and the Shannon (*H*) and Simpson (*D*) values are unitless index values representing relative abundances of species in a community. Statistically significant differences are represented by non-overlapping symbols (*N*=3). Error bars represent the standard error (*SE*) of the mean. (a) *S* was lower at the highest nutrient addition treatment than all other treatments and the control, though not significantly. (b, c) *H* and *D*, respectively, decreased with increasing nutrient availability, and values were lower at F10 than at F2 or F0.5. Differences in *H* and *D* between F10 and control plots tended toward significant (i.e., $P < 0.1$), indicated by significance coding in italics.

Chapter 2 – A mechanism of expansion: Arctic deciduous shrubs capitalize on increasing nutrient availability

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Abstract

Increasing nutrient availability, which is predicted to accompany Arctic warming, should have strong impacts on plant physiology, as Arctic tundra is a biome that is thought to be historically nitrogen (N)- and phosphorus- (P) limited. Thus, warming-induced nutrient enrichment may lead to shifts in leaf-level physiological properties and processes, affecting species and plant functional types differentially, with potential consequences for plant community dynamics and ecosystem function. To explore the physiological responses of Arctic tundra vegetation to increasing nutrient availability, we examined how a set of leaf nutrient and physiological characteristics of eight plant species, spanning four plant functional groups, respond to experimental N and P enrichment. Specifically, we examined a set of chlorophyll fluorescence measures related to photosynthetic efficiency, performance and stress, and two leaf nutrient traits (leaf %C and %N), across an experimental nutrient gradient at the Arctic Long Term Ecological Research site, located in the northern foothills of the Brooks Range, Alaska. In addition, we assessed the relationships between chlorophyll fluorescence measures and increasing leaf %N. We found significant differences in physiological and nutrient traits between species and plant functional groups, and we found that species within one functional group (deciduous shrubs) have significantly greater leaf %N at high levels of nutrient addition. In addition, we found positive, saturating relationships between leaf %N and chlorophyll fluorescence measures across all species. Our results highlight species-specific differences in leaf nutrient traits and physiology in this ecosystem. In particular, the effects of a gradient of nutrient enrichment were most prominent in deciduous plant species, the plant functional group known to be increasing in relative abundance with warming in this ecosystem. Overall, our results point to both complex and mixed responses of Arctic tundra vegetation to global-change induced nutrient enrichment.

Introduction

High-latitude ecosystems are warming rapidly, and Arctic tundra is predicted to be affected more by global environmental change than any other terrestrial ecosystem (IPCC 2013). The effects of Arctic warming include a deepening active (soil) layer, as well as increased soil nutrient mineralization, leading to the fertilization of a historically nitrogen (N)- and phosphorus (P)-limited landscape (Shaver and Chapin 1986, Chapin 1991). Greater nutrient availability is known to lead to shifts in plant community composition and physical structure with implications for key ecosystem functions such as carbon (C) and nutrient cycling (Rastetter et al. 1991, Hobbie and Chapin 1998, Myers-Smith et al. 2011). Existing fertilization experiments designed to explore the consequences of warming-induced nutrient enrichment of previously N- and P-limited Arctic tundra ecosystems have documented increases in net primary production (NPP) linked to shifts in plant community composition and physical structure over time (Shaver and Chapin 1986, Shaver et al. 1998, Boelman et al. 2003, Boelman et al. 2005). However, monitoring plant physiological responses to fertilization is equally important as plant traits often influence larger scale processes (Lavorel and Garnier 2002, Garnier et al. 2007). Understanding the relationship between a changing climate and plant physiology is particularly relevant in the Arctic as high-latitude ecosystems contain twice as much C as there is presently in the atmosphere (Zimov et al. 2006, Tarnocai et al. 2009) and between a quarter and a third of the C pools in the terrestrial biosphere (Carvalhais et al. 2014, Schimel et al. 2015).

Plant functional trait-based research is now a key component of community ecology (McGill et al. 2006), ultimately facilitating a more robust understanding of the relationship between species and the functioning of ecosystems (Wright et al. 2006, Messier et al. 2010), and the responses of plant communities to environmental change (Chapin 2003, Yang et al. 2011). In

addition, nutrient-limited ecosystems are often characterized by plant species that differ strongly in their ability to respond to alterations in resource availability. In nutrient-limited systems, shifts in plant diversity or responses of species-specific leaf physiology may precede any changes in ecosystem function (Aber et al., 1998). Therefore, monitoring species-specific, physiological mechanisms that perhaps underlie changes at the community and/or ecosystem level is critical to a comprehensive understanding of the impacts of global change on natural ecosystems, and is particularly important in ecosystems that are undergoing rapid environmental change.

At the leaf level, experimental nutrient enrichment in Arctic tundra has resulted in a variety of responses, including the stimulation of photosynthetic and respiratory rates (Baddeley et al. 1994, Chapin and Shaver 1996), unchanged or depressed photosynthetic/respiratory rates (Shaver et al. 1998, Bret-Harte et al. 2001), and the decoupling of leaf-level respiration and photosynthesis (Heskel et al. 2012), making it difficult to predict possible consequences for ecosystem C balance. However, at a fundamental level, all observed responses to nutrient fertilization represent a change in plant energetics, as the metabolic processes driving the biogeochemistry of C and N (e.g., photosynthesis and N metabolism) require energy inputs from the environment. Plant responses also result in the strategic allocation of biochemical resources to maximize net C gain (Bloom et al. 1985). For example, leaf N plays a key role in energy capture, first as a component of chlorophyll, the main light capturing pigment, and then as an essential part of the proteins comprising both the electron transport chain and the Calvin cycle (Field and Mooney 1986, Lambers et al. 2006). In addition, there is a well-known relationship between leaf N and photosynthesis or respiration (Field and Mooney 1986, Reich et al. 1998, Reich et al. 2008) that is widely used in global terrestrial biosphere and dynamic vegetation models (Cox et al. 2000, Kattge et al. 2009).

The relationship between leaf N and leaf metabolism suggests that leaf traits reflecting the status of energy capture and use should respond to nutrient fertilization and be used to monitor and predict plant and ecosystem processes and properties. Chlorophyll fluorescence has long been used as a non-invasive “probe of photosynthesis” and is widely used to evaluate photosystem II (PSII) photochemistry, linear electron flux, and even CO₂ assimilation in vivo (Baker 2008). While these chlorophyll fluorescence metrics have proven useful in thousands of studies, they have rarely been used in Arctic plants or included in studies of leaf traits, though recent work by Griffin et al. (2013) and Magney et al. (*In press*) highlight its efficacy in this system.

In this study, we examined how plant physiology differs between eight dominant species in low Arctic tundra exposed to nine years of incremental N and P enrichment. Specifically, we examined how multiple chlorophyll fluorescence measures and biochemical leaf traits (i.e., percent leaf C and N) of eight of the most abundant plant species at our study site, spanning four plant functional types, respond to a gradient of nutrient enrichment in northern Alaska. In addition, we evaluated the effects of variation in percent leaf N on other physiological traits across all species, as increasing soil nutrient availability should result in increasing leaf N content or greater interspecific variation in leaf N content. We hypothesized that deciduous species (i.e., deciduous shrub and forb species) would respond more strongly to nutrient enrichment than other plant functional types, due to relatively rapid leaf turnover and high rates of nutrient uptake, but that all species would show increases in leaf N and chlorophyll fluorescence traits with fertilization. In addition, we hypothesized that photosynthetic capacity and efficiency, as measured by a suite of chlorophyll fluorescence parameters, would be positively associated with leaf N content.

Methods

Site description and experimental design

All field sampling for this study took place during peak tundra leaf-out or greenness (July 10-25, 2015) across a long-term nitrogen (N) and phosphorus (P) enrichment experiment established in 2006 at the Arctic Long Term Ecological Research (ARC LTER) site, located at Toolik Lake in the northern foothills of the Brooks Range, Alaska (68°38'N and 149°43'W, 760 m a.s.l.). As we were interested in the effects of the magnitude of nutrient enrichment on the capacity for leaf-level physiology, we focused on examining a set of leaf properties and processes during the period of peak tundra greenness or leaf out which is approximately 30-35 days (Sweet et al. 2015) with an average growing season temperature of 10°C. As the growing season is short, we focused on conducting all field sampling during peak tundra leaf-out to ensure robust comparability of data between species and treatments.

The nutrient addition gradient is located on moist acidic tundra with soils comprised of 30–55 cm of a peaty organic and silty mineral layer, atop continuous permafrost. Each year following snowmelt, granular ammonium nitrate and triple superphosphate is distributed on the 5 × 20 m plots, corresponding to fertilization treatment. Treatment name corresponds to the amount of fertilizer applied at the beginning of each growing season: “CT”, a control that receives no fertilizer; “F0.5” (0.5 g N m⁻² yr⁻¹ + 0.25 g P m⁻² yr⁻¹); “F1” (1 g N m⁻² yr⁻¹ + 0.5 g P m⁻² yr⁻¹); “F2” (2 g N m⁻² yr⁻¹ + 1 g P m⁻² yr⁻¹); “F5” (5 g N m⁻² yr⁻¹ + 2.5 g P m⁻² yr⁻¹); and “F10” (10 g N m⁻² yr⁻¹ + 5 g P m⁻² yr⁻¹). The nutrient enrichment plots are replicated in a complete three-block design. Naturally occurring, thawing related nutrient enrichment is estimated to be roughly 0.3 g N m⁻² yr⁻¹, falling just below the lowest nutrient enrichment treatment in our study (Prager et al. 2017).

At each treatment plot, we measured a set of leaf physiological traits for eight species that account for roughly 75% of the plant cover in the experimental plots, spanning four plant functional types: deciduous shrubs, evergreen shrubs, forbs and grasses/sedges (here, “graminoid species”). We sampled two deciduous shrub species (*Betula nana* and *Vaccinium uliginosum*), two evergreen shrub species (*Ledum palustre* and *Vaccinium vitis-idaea*), two forb species (*Polygonum bistorta* and *Rubus chamaemorus*) and two graminoid species (*Carex bigelowii* and *Eriophorum vaginatum*).

Chlorophyll a fluorescence and leaf nutrient trait collection

As light energy absorbed by chlorophyll is diverted to photochemistry, lost as heat, or re-emitted as light, this technique allows us to assess the relative photosynthetic status of leaves without directly measuring leaf-level gas exchange (Bolhar-Nordenkamp and Oquist 1993). Well-developed methods exist to rapidly survey leaves and assess a wide variety of energetic parameters, including the maximum efficiency at which light absorbed by PSII is used for reduction of Quinone A (Q_A – the first electron acceptor of photosynthesis; F_v/F_m), the efficiency with which a trapped excitation energy can move beyond Q_A (ET_0/TR_0), and the fluorescence value at 300 μ s (K), which has been shown to reflect leaf N status (Schmitz and Maldonado-Rodriguez 2001). For each leaf sampled, we measured three chlorophyll fluorescence variables: (1) maximum quantum yield of primary photochemistry (F_v/F_m ratio), which is a reliable measure of plant stressors that affect photosystem II (PSII); (2) the efficiency (ET_0/TR_0) with which a trapped excitation energy can move an electron into the electron transport chain further than the Quinone A (Q_A); and (3) the fluorescence value at 300 μ s (K). We measured these three fluorescence traits using a modulated fluorometer model OS30p+ (Opti-Sciences, Hudson, NH, US) prior to harvesting leaves for nutrient analyses. We chose our three measures of

chlorophyll fluorescence because they are known to be indicative of photosynthetic stress and overall performance (i.e., F_v/F_m and ET_0/TR_0) or are thought to be most sensitive (i.e., K) to nutrient limitation (Schmitz and Maldonado-Rodriguez 2001, Strasser et al. 2004). In addition, these measures are presumably influenced by leaf nutrient status, and are comparable between species as well as between treatments (Cavender-Bares and A. Bazzaz 2004). Prior to each measurement, leaves were dark-adapted for at least 30 min to force reaction centers of PSII open (Strasser et al. 2000). Following dark acclimation, we administered a “JIP” Test protocol using a single strong pulse of light (i.e., $3500 \mu\text{mol m}^{-2} \text{s}^{-1}$) generated by an array of red LEDs (650 nm). We conducted one test per leaf and sampled five leaves (separate plants) per species per treatment plot per block. The five replicates were averaged to obtain plot level values for each species, yielding three observations per species per treatment.

In addition, we collected fresh leaf tissue from sunlit leaves used for chlorophyll fluorescence measurements, and we dried leaf samples at 65°C for 48 hours. All leaf samples were subsequently ground (Mini Bead Beater, Biospec) and processed at the Stable Isotope Core laboratory at Washington State University, where %C and %N were determined using automated dry combustion on a per mass basis (TruSpec CN, Leco Corporation, St. Joseph, MI). Three leaf samples were averaged to obtain plot level values at each block resulting in three independent observations for each species per block. For select species, we found no individuals in certain treatments or blocks, resulting in a reduction in the number of independent observations (see Tables 1-5). Leaf C and N reflect plant function because leaf C is thought to correlate with leaf mass per area, as leaves containing may high levels of vascular and sclerenchymatic are often correlated with high concentrations of leaf C (Reich 2014, de la Riva et al. 2016) and leaf N is essential in energy capture and use as discussed above. We consider all five leaf measures

sampled in this study to be functional traits as they represent morphological and physiological processes and properties which impact fitness indirectly via their effects on growth, reproduction and survival (Violle et al. 2007).

Statistical Methods

We ran a series of mixed effects models for each of our response variables (e.g., chlorophyll fluorescence measures and leaf nutrients) with treatment as a fixed effect and block as a random effect. Block did not have a significant effect, as determined by using simulation-based likelihood ratio tests, in any of our initial analyses. For all analyses, P -values < 0.05 were considered statistically significant. We also evaluated goodness of fit of additional non-linear models (i.e., Michaelis-Menten and logistic), using the Akaike Information Criterion (AIC) for model selection. In addition, we fit a series of nonlinear least squares models with percent leaf N as the predictor variable and chlorophyll fluorescence measures as response variables, using AIC for model selection. Finally, we conducted a principal component analysis to ordinate species by all five functional and physiological traits and by plant functional groups, delineating groups using a normal probability ellipse of 68%. All analyses were completed in R v. 3.2.1 (R CoreTeam 2015) using the cluster (Maechler et al. 2015), ggplot2 (Wickham 2009), lme4 (Bates et al. 2015), lsmeans (Lenth 2016), nlstools (Baty et al. 2015), nlsMicrobio (Baty and Delignette-Muller 2013), and stats (Team 2015) packages.

Results

Chlorophyll fluorescence and leaf nutrients by species

We found significant differences in leaf nutrients (i.e., leaf %C and %N) and fluorescence metrics (i.e., ET_0/TR_0 , F_v/F_m and K) between species and between plant functional types (e.g., deciduous shrubs, evergreen shrubs, forbs and graminoids), aggregating across all nutrient addition treatments. Overall, when considering all five leaf traits, individuals tended to group by plant functional type (Figure 1), and we found that 66% of the variation in the data could be explained by the first two principal components – 43% by the first axis and 24% by the second (Table 1). The main traits driving the first axis were %C and %N, in opposite directions. The second axis was primarily determined by F_v/F_m (Table 2). While we found clustering by plant functional group, deciduous shrubs and forbs had greater scatter than evergreen shrub species or graminoids (Figure 1).

In addition, when we compared each trait individually across species, we found significant differences between species in leaf %N. Specifically, we found that *Polygonum bistorta*, a forb species, had significantly higher leaf %N than all other species ($M=3.64$, $SD=0.81$), and that both forb species (i.e., *P. bistorta* and *Rubus chamaemorus*) and deciduous shrub species (*Betula nana* and *Vaccinium uliginosum*) had higher leaf %N than evergreen shrub species (*Rhododendron tomentosum* and *V. vitis-idaea*) and graminoid species (i.e., *Carex bigelowii* and *Eriophorum vaginatum*), and there were no differences between evergreen and graminoid species (Table 3). For leaf %C, *R. tomentosum* had significantly higher values than all other species except for the only other evergreen shrub species (*V. vitis-idaea*), and *B. nana* had higher %C than the two forb species and two graminoid species (Table 4). We found species-specific differences in F_v/F_m , with *B. nana*, *P. bistorta*, and *Ru. chamaemorus* exhibiting significantly higher ratios than the four evergreen shrub and graminoid species, but not higher than *V. uliginosum* nor each other (Table 5). For ET_0/TR_0 , we found fewer consistent patterns

(Table 6). For K, we found that *E. vaginatum* and *R. tomentosum* were not different from each other, but these two species displayed significantly lower K values than all other species (Table 7).

Nutrient enrichment impacts on functional groups and species

There were no significant differences in any leaf trait for species or functional groups with nutrient addition except for leaf %N. For deciduous shrub species, we found a significant, positive effect of nutrient addition on leaf %N, with 38% of the variance explained (Figure 2a; $P < 0.0001$). We did not find any significant differences between nutrient addition treatments and leaf %N for any of the other plant functional groups. However, when we examined plant functional groups based on their respective species, we found a significant effect of fertilization on leaf %N on both deciduous shrub species (i.e., *B. nana* and *V. uliginosum*), one forb species, *R. chamaemorus*, and one graminoid species (i.e., *E. vaginatum*). For *B. nana* we found that leaf %N increased with nutrient addition, and 72% of the variance explained (Figure 3a; $P < 0.0001$). For *V. uliginosum* we found that the slope was significant ($P < 0.05$) and 26% of the variance was explained (Figure 3a), for *R. chamaemorus*, 34% of the variance was explained (Figure 3b; $P < 0.05$), and 21% of the variance was explained for *E. vaginatum* (Figure 3c; $P < 0.05$).

Variation in leaf %N influences chlorophyll fluorescence measures

Given the variation in leaf N captured by incorporating multiple plant functional groups subjected to nutrient addition, we assessed the relationship between leaf %N and our three chlorophyll fluorescence traits. The relationship between F_v/F_m and %N was best described by a Michaelis-Menten model ($K_m = 0.50$, $SE=0.06$; $V_m = 0.86$, $SE=0.02$), indicating a positive,

saturation relationship between F_v/F_m and %N (Figure 4). The relationship between the fluorescence value at 300 μ s (K) and %N was also best described by a Michaelis-Menten model, indicating a positive, saturating relationship between K and %N ($K_m = 468.18$, $SE=31.15$; $V_m = 1.007$, $SE=0.22$). All of these relationships were found across all species, and not within a given species or functional group.

Discussion

As warming is occurring rapidly in Arctic tundra, predicting plant community and ecosystem-level responses to the effects of environmental change is of critical importance. The primary goals of this study to fertilizer addition were: 1) to assess how key functional traits differed among the eight most abundant plant species at a low Arctic tundra site, spanning four plant functional groups, 2) to determine how incremental nutrient addition affected these species and traits differentially, and 3) to explore the relationships between leaf nutrient and chlorophyll fluorescence traits across all eight species.

Inter-specific trait variation

There is a long-standing tradition in ecology of attempting to document and understand inherent morphological and physiological differences between plant species (Lambers et al. 2006) and the responses of such traits to environmental change (Lavorel and Garnier 2002, Suding et al. 2008). Here, we examined the most abundant plant species at our low Arctic tundra study site, and in support of our initial hypotheses, we found that species and functional groups differed fundamentally in their leaf traits regardless of nutrient addition. Not surprisingly, deciduous plant species (e.g., deciduous shrubs and forbs) demonstrated greater %N and F_v/F_m

ratios than evergreen species (here, both evergreen shrubs and graminoid species), and trait values were less constrained for deciduous species than they were for evergreen species (see Figure 1), with greater variation seen in deciduous shrub species attributable to their responses to nutrient enrichment. These findings are consistent with previous studies conducted across biomes, showing that species with relatively high leaf turnover tend to converge on rapid resource acquisition strategies compared to plant species with longer leaf lifespans (e.g., evergreen species) and conservative resource strategies (Grime et al. 1997, Diaz et al. 2004, Wright et al. 2004).

Nutrient enrichment impacts deciduous plant species

Across all nutrient-limited ecosystems, fertilization is known to impact leaf, community and ecosystem-level properties and processes, as nutrient enrichment leads to shifts in competitive interactions between plant species (Tilman 1984, 1987). In Arctic tundra, large-scale experimental studies have documented pronounced shifts in plant community composition over time with concurrent increases in net primary productivity (NPP), occurring in connection with increases in the abundance of deciduous woody shrub species and decreases in evergreen, grass/sedge, and moss cover (Shaver and Chapin 1986, Shaver et al. 1998, Boelman et al. 2003, Boelman et al. 2005). In addition, previous research at our study site found that six years of N and P fertilization comparable to the F10 treatment in our study resulted in increases in canopy leaf area of *Betula*, proposed as a plant morphological mechanism underlying increasing NPP (Bret-Harte et al. 2001). It is perhaps not surprising that we did not find strong responses in species within other functional groups, as some plants species are inherently less affected by fertilization and the alleviation of nutrient limitation as others. We may see a response in some

species, but not in others, as the traits that allow for the conservation of nutrients also tend to inhibit the acquisition and utilization of increasingly available nutrients (Chapin 1980, Chapin et al. 1986). However, our chlorophyll fluorescence measures, coupled with leaf N content, may suggest that the majority of the species that we evaluated are perhaps not nutrient limited.

Across the same nutrient addition gradient experiment used in this study, previous work found that deciduous shrub and forb species increased in relative abundance at high levels of nutrient addition, while evergreen shrub cover decreased. This community level response ultimately resulted in increased gross primary production (GPP) at high levels of nutrient addition (Prager et al. *In press*). Here, we show that two deciduous shrub species show increasing leaf N with nutrient enrichment that may contribute to this, suggesting both a physiological and morphological response of deciduous shrub species to nutrient enrichment. Previous work across this gradient examining two species, the current and historically dominant sedge species (*E. vaginatum*) and *B. nana*, showed that only leaf N of *B. nana* responded to high levels of fertilization (Heskel et al. 2012). Thus, our findings confirm previous findings and expand them to demonstrate that other deciduous shrub species respond similarly to increasing nutrient availability.

Ultimately, plant responses to nutrient addition may influence community and ecosystem dynamics via changes in leaf physiology and/or changes in relative abundance (Suding et al. 2005, Suding et al. 2008). While we did not find significant increases in leaf N for species in other functional groups, we did find that forb species had consistently high levels of leaf N, comparable to *Betula* leaf N at high levels of fertilization. Therefore, though we did not see a strong response in foliar N of forb species, canopy N would increase disproportionately with

increasing nutrient addition due to relatively high overall levels of leaf N combined with increased percent cover of these species.

Positive, saturating relationships between leaf nitrogen and chlorophyll fluorescence

All of the biochemical processes of photosynthesis require nitrogenous compounds – from proteins that catalyze the reactions of CO₂ fixation to those produced by light-driven electron transport. Thus, the degree of nitrogenous investment in leaves is inherently linked to complex physiological processes associated with photosynthesis and C gain (Field and Mooney 1986, Lambers et al. 2006). Here, given the variation generated by inter-specific differences in N use across species and functional groups, and by experimental nutrient fertilization, we found positive, saturating relationships between leaf %N and chlorophyll fluorescence traits across all species and plots. As chlorophyll fluorescence is often used to assess photosynthetic efficiency and plant performance (Cavender-Bares and A. Bazzaz 2004), these positive, saturating relationships demonstrate the importance of leaf N in modulating plant function and show that inherently high levels of foliar N, or the ability to acquire and utilize greater N availability, appear to be advantageous in this system. While these relationships are perhaps not surprising, given known relationships between foliar N and photosynthesis, the saturating nature of the relationship between these traits has not yet been described for Arctic tundra plant species.

Acknowledgements

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Tables

Table 2.1 1

Table 1. Plant Trait Principal Component Analysis

Principal Component Axis	PC1	PC2	PC3	PC4	PC5
Proportion of Variance Explained	0.43	0.24	0.17	0.13	0.04
Cumulative Variance Explained	0.43	0.66	0.83	0.96	1

Table 2.2 1

Table 2. Plant Trait Principal Component Analysis Loadings

Trait	PC1	PC2	PC3	PC4	PC5
K	-0.47	0.33	-0.50	0.48	0.44
Fv/Fm	-0.61	-0.23	-0.07	0.15	-0.74
ETo.TR	-0.26	-0.79	0.26	0.13	0.47
%N	-0.50	0.14	-0.05	-0.83	0.21
%C	0.29	-0.44	-0.82	-0.21	-0.06

Table 2.3 1

Table 3. Results from Tukey's Honest Significance Difference (HSD) test on Leaf %N across species.

Tukey's HSD Comparisons (Leaf %N)											
Group	<i>n</i>	<i>SD</i>	Mean	<i>Betula nana</i>	<i>Vaccinium uliginosum</i>	<i>Polygonum bistorta</i>	<i>Rubus chamaemorus</i>	<i>Carex bigelowii</i>	<i>Eriophorum vaginatum</i>	<i>Ledum palustre</i>	<i>Vaccinium vitis-idea</i>
<i>Betula nana</i>	18	0.81	3.07	-							
<i>Vaccinium uliginosum</i>	14	0.41	2.28	<.001	-						
<i>Polygonum bistorta</i>	18	0.41	3.64	<.001	<.001	-					
<i>Rubus chamaemorus</i>	12	0.23	2.41	<.001	.975	<.001	-				
<i>Carex bigelowii</i>	18	0.27	2.26	<.001	1	<.001	.970	-			
<i>Eriophorum vaginatum</i>	17	0.26	2.15	<.001	.994	<.001	.637	.991	-		
<i>Ledum palustre</i>	18	0.09	1.96	<.001	.399	<.001	<.05	.287	.846	-	
<i>Vaccinium vitis-idea</i>	18	0.14	1.95	<.001	.388	<.001	<.05	.846	.836	1	-

Table 2.4 1

Table 4. Results from Tukey's Honest Significance Difference (HSD) test on Leaf %C across species.

Group	n	SD	Mean	Tukey's HSD Comparisons (Leaf %C)								
				<i>Betula nana</i>	<i>Vaccinium uliginosum</i>	<i>Polygonum bistorta</i>	<i>Rubus chamaemorus</i>	<i>Carex bigelowii</i>	<i>Eriophorum vaginatum</i>	<i>Ledum palustre</i>	<i>Vaccinium vitis-idea</i>	
<i>Betula nana</i>	18	5.47	48.18	-								
<i>Vaccinium uliginosum</i>	14	3.84	47.69	.999	-							
<i>Polygonum bistorta</i>	18	3.01	42.68	<.001	<.005	-						
<i>Rubus chamaemorus</i>	12	1.32	41.60	<.001	<.001	.993	-					
<i>Carex bigelowii</i>	18	3.39	44.01	<.02	.101	.959	.644	-				
<i>Eriophorum vaginatum</i>	17	4.38	44.56	.078	.269	.799	.394	.999	-			
<i>Ledum palustre</i>	18	3.23	53.62	<.001	<.001	<.001	<.001	<.001	<.001	-		
<i>Vaccinium vitis-idea</i>	18	2.77	50.46	.576	.408	<.001	<.001	<.001	<.001	.173	-	

Table 2.5 1

Table 5. Results from Tukey's Honest Significance Difference (HSD) test on F_v/F_m across species.

Group	n	SD	Mean	Tukey's HSD Comparisons (F _v /F _m)							
				<i>Betula nana</i>	<i>Vaccinium uliginosum</i>	<i>Polygonum bistorta</i>	<i>Rubus chamaemorus</i>	<i>Carex bigelowii</i>	<i>Eriophorum vaginatum</i>	<i>Ledum palustre</i>	<i>Vaccinium vitis-idea</i>
<i>Betula nana</i>	18	0.04	0.74	-							
<i>Vaccinium uliginosum</i>	14	0.04	0.70	.120	-						
<i>Polygonum bistorta</i>	18	0.01	0.78	.399	< .001	-					
<i>Rubus chamaemorus</i>	12	0.03	0.74	.999	.406	.309	-				
<i>Carex bigelowii</i>	18	0.03	0.68	<.001	.822	<.001	<.009	-			
<i>Eriophorum vaginatum</i>	17	0.05	0.65	<.001	<.030	<.001	<.001	.437	-		
<i>Ledum palustre</i>	18	0.04	0.64	<.001	<.004	<.001	<.001	.166	.999	-	
<i>Vaccinium vitis-idea</i>	18	0.06	0.68	< .001	.738	<.001	<.006	.999	.542	.233	-

Table 2.6 1

Table 6. Results from Tukey's Honest Significance Difference (HSD) test on ET₀/TR₀ across species.

Group	n	SD	Mean	Tukey's HSD Comparisons (ET ₀ /TR ₀)							
				<i>Betula nana</i>	<i>Vaccinium uliginosum</i>	<i>Polygonum bistorta</i>	<i>Rubus chamaemorus</i>	<i>Carex bigelowii</i>	<i>Eriophorum vaginatum</i>	<i>Ledum palustre</i>	<i>Vaccinium vitis-idea</i>
<i>Betula nana</i>	18	0.05	0.60	-							
<i>Vaccinium uliginosum</i>	14	0.07	0.62	.979	-						
<i>Polygonum bistorta</i>	18	0.04	0.69	< .001	< .002	-					
<i>Rubus chamaemorus</i>	12	0.03	0.65	.100	.621	.469	-				
<i>Carex bigelowii</i>	18	0.05	0.57	.793	.259	< .001	< .001	-			
<i>Eriophorum vaginatum</i>	17	0.05	0.63	.649	.997	< .01	.929	< .05	-		
<i>Ledum palustre</i>	18	0.04	0.64	.288	.925	< .05	.996	< .005	.999	-	
<i>Vaccinium vitis-idea</i>	18	0.05	0.65	.050	.537	.285	1	< .001	.905	.995	-

Table 2.7 1

Table 7. Results from Tukey's Honest Significance Difference (HSD) test on K across species.

Group	n	SD	Mean	Tukey's HSD Comparisons (K)							
				<i>Betula nana</i>	<i>Vaccinium uliginosum</i>	<i>Polygonum bistorta</i>	<i>Rubus chamaemorus</i>	<i>Carex bigelowii</i>	<i>Eriophorum vaginatum</i>	<i>Ledum palustre</i>	<i>Vaccinium vitis-idea</i>
<i>Betula nana</i>	18	43.72	337.91	-							
<i>Vaccinium uliginosum</i>	14	69.81	367.12	.665	-						
<i>Polygonum bistorta</i>	18	32.15	381.88	.107	.988	-					
<i>Rubus chamaemorus</i>	12	29.13	341.67	.999	.870	.311	-				
<i>Carex bigelowii</i>	18	34.52	367.46	.570	1	.984	.825	-			
<i>Eriophorum vaginatum</i>	17	37.05	213.12	<.001	<.001	<.001	<.001	<.001	-		
<i>Ledum palustre</i>	18	48.88	236.44	<.001	<.001	<.001	<.001	<.001	.827	-	
<i>Vaccinium vitis-idea</i>	18	65.81	362.84	.760	.999	.928	.930	.999	<.001	<.001	-

Figures

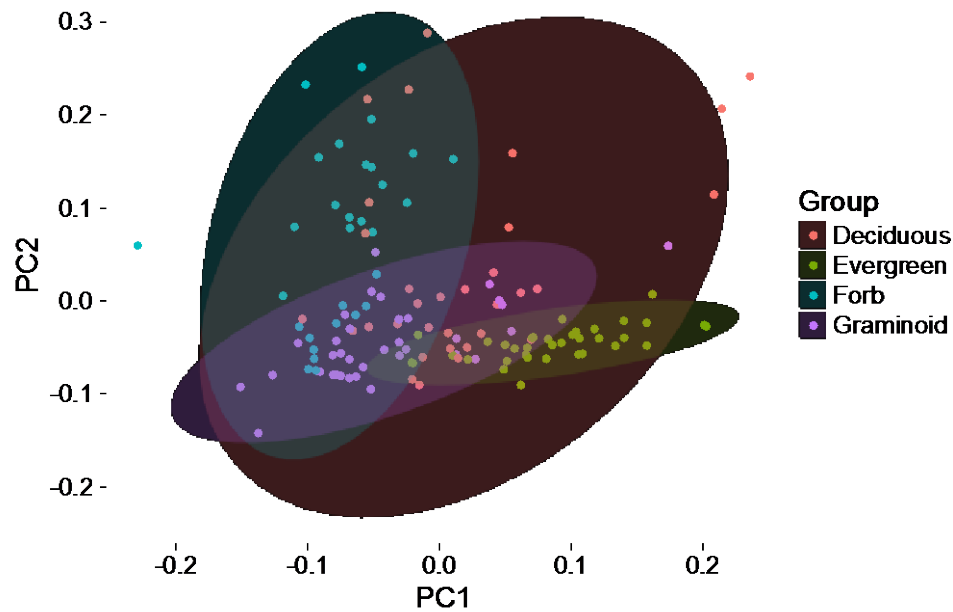


Figure 2.1 1

Figure 1. Principal component analysis of eight study species, grouped by plant functional type, by two leaf nutrients (leaf %C and %N) and three chlorophyll fluorescence metrics (ET_o/TR_o , F_v/F_m , and K). Ellipses are normal data probability ellipses, using a normal probability of 68%. We found that 66% of the variation in the data could be explained by the first two principal components – 43% by the first axis and 24% by the second – and the main traits driving the first axis were %C and %N, in opposite directions. The second axis was primarily determined by F_v/F_m . We found that individuals grouped by species and plant functional type (e.g., deciduous shrubs, evergreen shrubs, forbs and graminoids), but that deciduous shrubs and forbs had greater scatter than evergreen shrub species or graminoids.

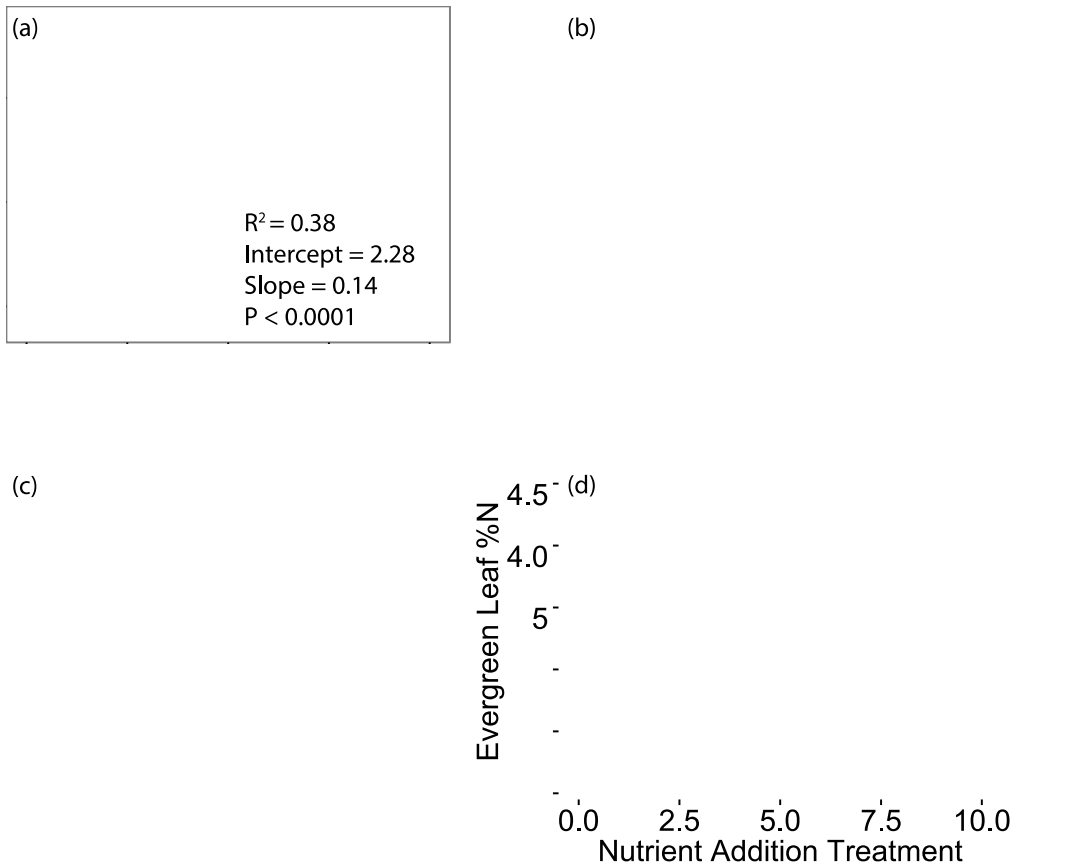


Figure 2.2 1

Figure 2. Percent leaf N by plant functional type across a gradient of nutrient (N and P) addition. Points are mean leaf %N and a solid, gray linear regression line is shown. (a) For deciduous shrub species, leaf %N increased with nutrient addition, and 38% of the variance was explained ($p < 0.0001$). (b-d) There were no significant effects of nutrient addition on any other plant functional group.

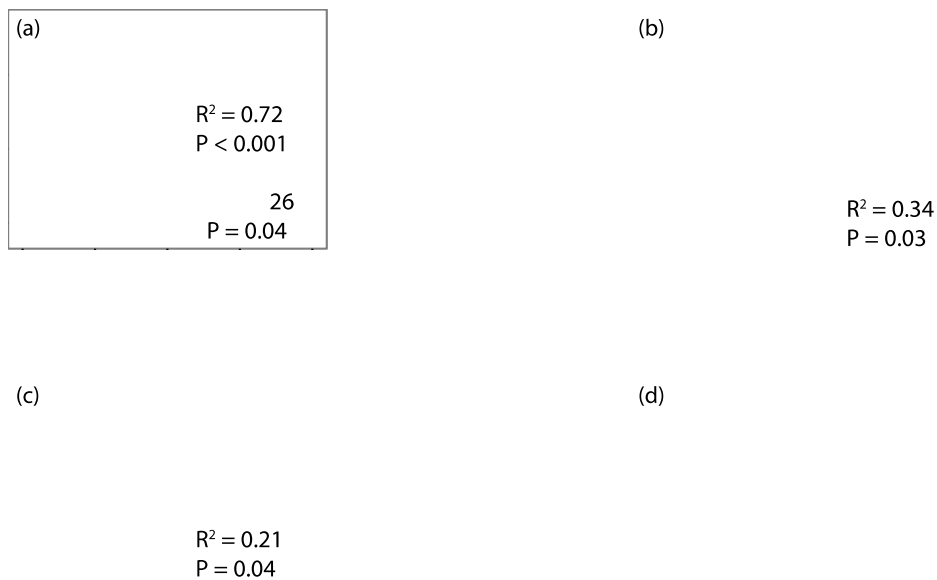


Figure 2.3 1

Figure 3. Linear regressions depicting the relationship between nutrient addition and leaf %N for individual species within plant functional groups. Points represent mean %N, and solid regression lines are shown. (a) For both *Betula nana* (BETNAN) and *Vaccinium uliginosum* (VACULI), leaf %N increased with nutrient addition, with a stronger response of *B. nana* to addition than for *V. uliginosum*. For *B. nana*, 72% of the variance in leaf %N was explained ($p < 0.001$), and for *V. uliginosum* 26% of the variance was explained ($p < 0.05$). (b) For forb species, one species, *Rubus chamaemorus* (RUBCHA), leaf %N increased with nutrient addition, and 34% of the variance was explained. (c) For one graminoid species, *Eriophorum vaginatum* (ERIVAG), leaf %N increased with nutrient enrichment, and 21% of the variance was explained. (d) There was no impact of increasing nutrient availability on either evergreen shrub species ($p > 0.05$).

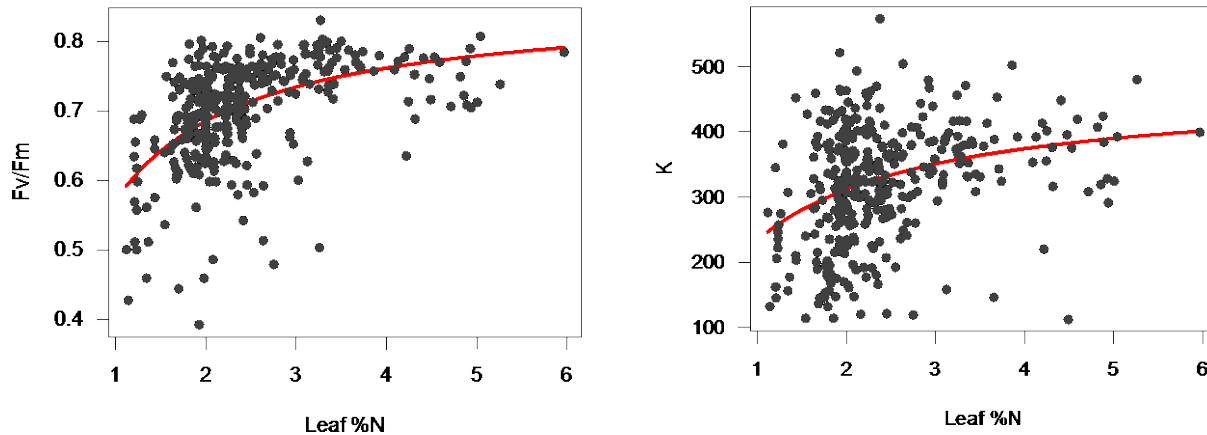


Figure 2.4 1

Figure 4. Significant relationships between chlorophyll fluorescence measures and percent leaf nitrogen (N). Optimum F_v/F_m values may vary from species to species, but generally fall within 0.79-0.84. (a) Relationship between maximum quantum yield of primary photochemistry (F_v/F_m ratio), across all species, and leaf %N. The relationship between F_v/F_m and leaf %N was best described by a Michaelis-Menten model ($K_m = 0.50$, $SE=0.06$; $V_m = 0.86$, $SE=0.02$), as determined by the Akaike Information Criterion (AIC), indicating a positive, saturating relationship between F_v/F_m ratio and leaf %N. (b) Relationship between the fluorescence value at 300 μs (K) and leaf %N was best described by a Michaelis-Menten model, indicating a positive, saturating relationship between K and leaf % ($K_m = 468.18$, $SE=31.15$; $V_m = 1.007$, $SE=0.22$).

Chapter 3 – Long-term nutrient enrichment differentially impacts multiple dimensions of biodiversity

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Abstract

Globally, biodiversity losses due to environmental change are escalating, resulting in the impairment of key ecosystem processes and properties. Therefore, understanding how multiple dimensions of diversity (e.g., functional, phylogenetic, taxonomic) respond to long-term global change is paramount, particularly across ecosystems that are experiencing rapid environmental change. While biodiversity is generally understood to be a complex entity, little attention has been given to examining multiple dimensions of diversity simultaneously, possibly underestimating the magnitude of the effects of global change on communities and ecosystems. Here, we examined how three dimensions of biodiversity (functional, phylogenetic and taxonomic) respond to long-term nutrient enrichment (i.e., > 20 years) in Arctic tundra, a biome warming twice as fast as the global average. Overall, we found that long-term nitrogen (N) and phosphorus (P) addition led to significant declines in all three dimensions of diversity, and that these effects persist through time. In addition, we found that the effects of N and P addition on two dimensions of diversity that capture important organismal traits – functional dispersion (F_{Dis}) and mean pairwise distance (MPD), a measure of phylogenetic diversity – increased with time. Our results highlight the importance of measuring and monitoring multiple dimensions of diversity as global change does not impact species and species' traits uniformly. Furthermore, as functional diversity (FD) and phylogenetic diversity (PD) are known to be strong predictors of ecosystem processes (e.g., productivity and nutrient cycling) and properties (e.g., stability and resilience), the differential impacts of nutrient enrichment on FD and PD have potentially significant implications for the long-term maintenance of ecosystem functioning in this biome.

Introduction

The indirect and direct effects of climate change are leading to significant declines in biodiversity and the impairment of ecosystem functioning worldwide (McMahon et al. 2011). As biodiversity losses are escalating, accurately measuring changes in relevant dimensions of biodiversity (e.g., taxonomic, functional, phylogenetic, etc.) in order to best predict the potential ecological consequences of global environmental change is paramount (Naeem et al. 2016a, Seddon et al. 2016), and, consequently, monitoring the responses of biodiversity to global change is under intense investigation (Pereira et al. 2010, Dawson et al. 2011). Furthermore, as biodiversity is now generally accepted to positively influence larger scale ecosystem processes and properties (Tilman et al. 1997b, Loreau et al. 2001, Naeem et al. 2012), accurately predicting the impacts of global change on ecosystems relies on an accurate and comprehensive understanding of the dimensions of biodiversity. What constitutes a dimension of biodiversity varies, with taxonomic, functional, and phylogenetic the most frequently used (Naeem et al. 2016b). Other dimensions include genetic, genomic, and measures of trophic- or community-web properties, to name just a few, and may be further refined by the incorporation of relative abundance or reference to temporal or spatial scales, such as turnover or landscape diversity. Examining multiple dimensions of diversity – combining an understanding of taxonomic (species), functional (trait) and phylogenetic diversity – may help better predict ecosystem consequences of realistic species losses.

While biodiversity is understood to be multidimensional, very few studies go beyond examining a single dimension of diversity (Naeem et al. 2016b). Recent work suggests that using functional diversity (FD) – quantifying the type, range and relative abundance of organismal traits in ecological communities – rather than taxonomic diversity (TD) can improve our ability to develop a mechanistic understanding of how and why biodiversity loss impacts

ecosystem functioning and the services derived from these functions (Petchey et al. 2004, de Bello et al. 2009, Cardinale et al. 2012). Furthermore, recent work suggests that additional dimensions of diversity, beyond taxonomic and functional diversity, are important to consider as phylogenetic diversity (PD) – a measure of evolutionary history in a community – may explain significantly more variation in primary productivity than species or functional group richness (Cadotte et al. 2008, Flynn et al. 2011, Cadotte 2013) because functional traits are often conserved over the phylogeny. In addition, PD may influence processes and properties beyond ecosystem productivity, such as stability (Cadotte et al. 2012) and multitrophic interactions (Dinnage et al. 2012). Furthermore, PD is an important dimension to consider as species losses are often non-random and closely related species presumably share similar traits making them more or less predisposed to be affected by environmental change (Willis et al. 2008). Finally, PD captures the intrinsic conservation value of communities (Winter et al. 2013, Frishkoff et al. 2014). Thus, using multi-dimensional diversity – combining an understanding of TD, FD and PD – is an important step toward better predictions of the consequences of realistic species losses, particularly in ecosystems that are undergoing rapid environmental change.

High-latitude ecosystems are warming quickly and Arctic tundra ecosystems are predicted to be strongly impacted by increasing surface air temperatures (IPCC 2013). The effects of warming on Arctic ecosystems include a deepening soil layer during the growing season as well as increased soil nutrient mineralization (Shaver and Chapin 1991). Because Arctic ecosystems are thought to be nitrogen (N) and phosphorus (P) limited, increasing N and P availability accompanying warming likely reduces plant diversity and affects interspecific competition, advantaging plant species differentially, favoring species with a greater capacity to access and utilize a growing soil nutrient pool (Shaver and Chapin 1986, Chapin 1991). For

example, increasing nutrient availability across tundra ecosystems is known to lead to shifts in plant community composition and physical structure with implications for ecosystem functions such as carbon (C) cycling (Rastetter et al. 1991, Hobbie and Chapin 1998, Myers-Smith et al. 2011).

Existing nutrient addition experiments designed to explore the consequences of warming-induced nutrient enrichment, and the alleviation of nutrient limitation in Arctic tundra, have demonstrated increases in net primary production (NPP) and in deciduous shrub cover with concurrent decreases in evergreen shrub and grass/sedge cover (Shaver and Chapin 1986, Shaver et al. 1998, Boelman et al. 2003, Boelman et al. 2005). While great attention has been given to the impacts of warming-related nutrient enrichment on plant functional group relative abundance and ecosystem productivity, little consideration has been given to the impacts of long-term nutrient addition on multiple dimensions of Arctic plant diversity that capture key relationships between species and are potentially indicative of important community and ecosystem dynamics, processes and properties.

Here, we examine how long term experimental nutrient enrichment (i.e., > 20 years) impacts multiple dimensions of Arctic tundra plant diversity and we evaluate how these effects vary through time. Specifically, we focus on understanding the impact of high levels of N and P addition (i.e., 10g/m² N as NH₄NO₃ and 5 g/m² P as triple superphosphate) on two measures of plant TD, species richness and one abundance-weighted measure (the Shannon Index), one measure of FD that captures species abundances and the distribution of functional traits within the trait space of a community (functional dispersion), and one abundance-weighted measure of PD (mean pairwise distance, MPD). We hypothesized that nutrient addition would lead to reductions in all dimensions of diversity, but reductions in diversity would be greatest for

measures of diversity that encompass both relative abundance and organismal traits (e.g., FD and PD). Furthermore, we hypothesized that the difference in diversity levels between control plots and nutrient addition plots would increase through time given shifts in interspecific interactions driven by changes in nutrient availability.

Methods

Site description and experimental design

All field sampling for this study took place during the period of peak tundra greenness from 1996-2010 across long-term global change experiments established in 1989, 1996 and 1997 at the Arctic Long Term Ecological Research (ARC LTER) site, located at Toolik Lake in the northern foothills of the Brooks Range, Alaska (68°38'N and 149°43'W, 760 m a.s.l.). The growing season at the ARC LTER site spans 10-12 weeks, beginning in early to mid-June, with an average growing season temperature of 10°C. The LTER moist acidic tussock (MAT) global change experiment was established in 1989 and is comprised of four blocks of 5 x 20 m plots with randomly assigned environmental change treatments within each block. Treatments include a control (CT) and nitrogen (N) plus phosphorus (P) addition. Granular fertilizer comprised of 10g/m² N (NH₄NO₃) and 5 g/m² P (triple superphosphate) is added annually following snowmelt. In addition, to evaluate the impact of herbivory, exclosure plots were set up at the MAT site in July 1996 on reserved 5 x 20 m plots within the existing four-block design of the 1989 MAT experimental plots. In each block, two fenced plots were established: a plot with no fertilizer and a plot with annual fertilization treatments as described above. Thus, the treatments established are no fence, no fertilizer (NFCT); small mesh fence, no fertilizer (SFCT); large mesh fence, no fertilizer (LFCT); no fence, N plus P (NFNP); small mesh fence, N plus P (SFNP); and large

mesh fence, N plus P (LFNP). Experimental plots were established at the LTER moist non-acidic tussock (MNT) site in 1997 following the same methods describe above, replicated in a three-block design. As we were primarily interested in the effects of long-term nutrient enrichment, we consider all control or NP addition plots, regardless of site (MAT or MNT), as one group. Thus, for this study, we evaluated the control (CT) and N plus P (NP) addition plots at the MAT and MNT sites and the control and NP plots without fencing added to the MAT experiment in 1996.

Plant percent cover and trait data

Following standard protocol outlined in Gough and Hobbie (2003), eight 1m² permanent quadrats are sampled annually in late July in each treatment plot per block. Within each quadrat, aerial percent cover of all vascular plants species was recorded as well as bare ground, lichen and moss cover. To standardize cover among plots, all percent cover values were summed within each plot, and then each value was divided by the total resulting in a percent cover value standardized to 100%. All percent cover and trait data were available through the LTER data portal(Gough 2015b, d, f, e, a, c, Gough and Hobbie 2015b, a).

In 2000, 2006 and 2012 four quadrats were randomly located along line transects in each of the replicate blocks of each treatment. Quadrats were then harvested and sorted into species and tissue type within 24 hours, and all leaf tissue samples were then dried at 65 C and weighed. Samples were aggregated by block and subsequently ground in a Wiley Mill and percent C and N were analyzed using an elemental analyzer at the University of Nebraska, Lincoln (ECS 4010, Costech Analytical, Valencia, California). Further detail on biomass harvests and subsequent plant nutrient analyses are provided in Shaver and Chapin (1991).

Dimensions of biodiversity

Functional diversity (FD) Functional diversity (FD) was trait based (e.g., as opposed to functional groups) and was measured given the distribution of plant functional traits within trait-space. We calculated functional dispersion (F_{Dis}) implemented in the R Package *FD* (Laliberte and Legendre 2010). F_{Dis} is the mean distance of individual species to the centroid of all species in the community and accounts for relative abundances by computing distances of individual species to a weighted centroid (Laliberte and Legendre 2010), capturing the degree to which species are close to the center of functional trait space or closer to the edges of the multidimensional volume. F_{Dis} was calculated using standardized values of two functional traits: leaf carbon content (%C) and leaf nitrogen content (%N). Leaf C reflects fundamental components of overall plant function as it is thought to correlate with leaf mass per area (Reich 2014, de la Riva et al. 2016), and leaf N plays a key role in energy capture, first as a component of chlorophyll, the main light capturing pigment, and then as an essential part of the proteins comprising both the electron transport chain and the Calvin cycle (Field and Mooney 1986, Lambers et al. 2006). Furthermore, there is a well-documented relationship between leaf N and photosynthesis and respiration (Field and Mooney 1986, Reich et al. 1998, Reich et al. 2008). Because trait data were only available in years when biomass harvests were conducted, we matched trait data sampled in 2000 for community data sampled from 1996-2002, trait data sampled in 2006 for community data sampled from 2003-2009, and trait data collected in 2012 for community data sampled from 2010-2012.

Phylogenetic diversity Phylogenetic diversity (*PD*) was estimated using an abundance-weighted metric, mean pair-wise distance (*MPD*), previously shown to be the best predictor of

ecosystem function (Cadotte et al. 2008), calculated using the *picante* package in R (Kembel et al. 2010) with phylogenetic relationships and branch lengths obtained from a locally stored phylogeny in *phylomatic* (Zanne et al. 2014), processed using Phylomatic V3 (Webb et al. 2008) and the *ape* (Paradis et al. 2004) and *phytools* (Revell 2012) packages in R. MPD is the mean sum of the phylogenetic branch lengths between all species pairs within a community.

Taxonomic diversity (TD) We calculated species richness (S), the number of species in a community, and the Shannon (H) index that reflects evenness and dominance of species within a community. All measures of taxonomic diversity were calculated using the *vegan* package (Okasen et al. 2015) in R v. 3.2.1 (R Core Team 2015).

Statistical Methods

We ran a series of linear mixed effects models for each of our response variables (i.e., measures of functional, phylogenetic and taxonomic diversity and the differences between control plots and nutrient addition plots for each of the four diversity metrics) with treatment and year as fixed effects and site as a random effect. In addition, we calculated correlation coefficients between our diversity measures. All analyses and data visualizations were completed in R v. 3.2.1 (R CoreTeam 2015) using the *ggplot2* (Wickham 2009), *lme4* (Bates et al. 2015), *lsmeans* (Lenth 2016), and *stats* (Team 2015) packages.

Results

We found that control plots consistently exhibited higher levels of diversity than nutrient addition plots, despite relatively comparable initial levels of diversity. However, for all measures of diversity in both control and nutrient addition plots, diversity declined through time with the exception of functional dispersion (F_{Dis}) in control plots, which increased with time

(Figure 1a-d). The change in species richness (S) through time was significant (Figure 1a) and 52% of the variance was explained in control plots and 41% of the variance was explained for S in nutrient addition plots. For the Shannon Index (H), relationships were significant, and 38% of the variance was explained in control plots versus 59% of the variance in nutrient addition plots (Figure 1b). For F_{Dis} , 38% of the variation was explained in control plots and 48% of the variance was explained in nutrient addition plots (Figure 1c). Finally, for MPD, 33% was explained in control plots and 71% of the variance was explained in nutrient addition plots (Figure 1d).

Mean S in control plots dropped from 25.33 (SD 2.08) in year two to 12.25 (SD 0.69) in year 10 to 12.01 (0.82) in year 21, compared to S in nutrient enrichment plots which was 25.50 (SD 0.71) in year two, dropping to 5.29 (SD 1.25) in year 10 and then to 4.33 (SD 0.58) in year 21. Mean H values in control plots started at 2.76 in year two, dropping to 2.09 (SD 0.07) and did not change from year 10 to 21 (i.e., $M=2.10$, $SD=0.09$ in year 21). In nutrient addition plots, mean H in year two was 2.67 (SD 0.02), dropping substantially to 0.96 (SD 0.31) in year 10 and then to 0.89 (SD 0.10) in year 21. Mean F_{Dis} in control plots was 0.33 (SD 0.005) in year two, increasing slightly in year 10 (i.e., $M=0.35$, $SD=0.01$), and in year 21 mean F_{Dis} was 0.42 (SD 0.01). In nutrient addition plots, mean F_{Dis} in year two was 0.30 (SD 0.02), dropping to 0.19 (SD 0.08) in year 10, and then to 0.10 (SD 0.05) in year 21. Finally, mean MPD in control plots in year two was 253.22 (SD 1.82), 238.83 (SD 4.47) in year 10, dropping to 234.21 (SD 3.46) in year 21. For nutrient addition plots, MPD was 256.25 (SD 1.99) in year 2, dropping to 175.15 (SD 21.26) in year 10 and then to 127.49 (SD 14.62) in year 21.

When we explored the difference in our four measures of diversity between control and nutrient addition plots, we found that the effect of nutrient addition on diversity remained the

same for two measures of diversity (i.e., S and H) and changed through time for two measures of diversity (i.e., F_{Dis} and MPD). For F_{Dis} , the difference between control plots and nutrient addition plots increased as the experiment went on and 27% of the variance was explained ($P < 0.002$; Figure 2c). Likewise, the difference between MPD in control plots and nutrient addition plots increased through time, and 15% of the variance was explained ($P < 0.007$; Figure 2d).

Discussion

Our results indicate that increased nutrient availability results in a reduction in biodiversity, regardless of the dimension of diversity examined; however, the magnitude of the effect varies depending on the observed measure of diversity. As biodiversity is known to influence the functioning of ecosystems, especially when considering dimensions of diversity that capture key functional traits and evolutionary relationships, monitoring and predicting the impacts of global change on multidimensional biodiversity through time is paramount. Furthermore, our findings highlight the importance of considering biodiversity to be multidimensional, as the examination of a single dimension of diversity may obscure potential impacts of global change on plant the functioning of plant communities.

Nutrient addition reduces taxonomic diversity

Across 21 years, we found that both species richness (S) and an abundance-weighted measure of taxonomic diversity (TD), the Shannon Index (H) declined through time. However, we found that no more species were lost from communities, due to nutrient enrichment, in year 21 than in year 10. Furthermore, the relative effect of nutrient addition on TD did not change through time – the difference in measures of TD in nutrient addition plots and control plots did not increase with time as they did for functional (FD) and phylogenetic diversity (PD). While

we also found a reduction in S and H in control plots, presumably due to the effects of naturally occurring warming, the difference between the control and addition plots remained the same. These findings are in keeping with both empirical and modeling work across the globe examining the impacts of increasing nutrient availability (often, N deposition) on TD, finding significant shifts in plant community composition and reductions in overall species richness (Bobbink et al. 2010). In Arctic tundra, studies examining the effects of annual N and P additions, some of which conducted on these plots, have demonstrated increased cover of vascular plants, decreased cover of bryophytes and lichens, and associated overall declines in species richness (Shaver and Chapin 1995, Gordon et al. 2001, Gough and Hobbie 2003, Soudzilovskaia and Onipchenko 2005).

Nutrient enrichment decreases functional dispersion

In our study, functional dispersion (F_{Dis}) decreased with nutrient addition at a more rapid rate than for either measure of TD. Interestingly, F_{Dis} increased in control plots through time, indicating that the distribution of functional traits within communities became more evenly spaced, farther away from the centroid of the community through time, possibly indicating a decrease in functional redundancy or an increase in the potential for niche complementarity. As the relative importance of plant diversity as a driver of ecosystem functioning has been shown to increase over time (Cardinale et al. 2012) in studies examining the connections between biodiversity and ecosystem function, these findings have potential consequences for larger scale ecosystem processes and properties. Often, communities with high degrees of functional divergence or dispersion, indicating a high degree of niche differentiation, correspond to high levels of ecosystem function, and high species turnover may allow for communities of greater

diversity to support higher levels of productivity via complementarity through time as species that are rare in some years may become dominant in others, driving biomass production in those years (Allan et al. 2011).

In addition, we found that increased nutrient availability led to decreased F_{Dis} and that the effect of nutrient addition on F_{Dis} increased through time, in contrast with what we found for TD. We found that the relative effects of nutrient addition on F_{Dis} grew through time, due in part to small increases in F_{Dis} in control plots and simultaneous decreases in F_{Dis} in nutrient enrichment plots. These findings are perhaps not surprising as species' sensitivity to disturbance is modulated by key organismal traits (Keinath et al. 2017), and disturbance has been shown to impact measures of FD in other systems. For example, a meta-analysis examining the effects of land-use change on FD of multiple taxa found that land-use change associated declines in FD are often greater than expected for mammal, bird and non-herbaceous plant taxa (Flynn et al. 2009). Our finding that the difference in FD between control and nutrient addition plots increased through time is particularly important both in the context of the perceived positive relationship between biodiversity and ecosystem function and in light of our finding that the difference between TD in control and nutrient addition plots did not appear to change through time.

Phylogenetic diversity declines sharply with nutrient addition

Similarly, we found that mean pairwise distance (MPD), a measure of phylogenetic diversity (PD), decreased with nutrient addition, and that MPD continued to decline in nutrient enrichment plots, relative to control plots, through time. Responses of PD to anthropogenic disturbance have been examined across taxa, usually with an attention to the responses of PD to land conversion or other local environmental disturbances (e.g., species invasion). In such

studies, disturbance has been shown to lead to the persistence of subsets of closely related clades, resulting in declines in PD (Dinnage 2009, Helmus et al. 2010). In addition, PD of flowering plants in the northeastern United States has been shown to respond strongly to warming, as species that are declining in abundance with warming are more closely related than expected by chance and species with flowering times that do not track seasonal temperature shifts have declined in abundance over the past 100 years (Willis et al. 2008). In our study, increased nutrient availability may act as an ecological filter, ultimately resulting in declines in PD.

Conclusion

Despite a long-standing effort to monitor and predict the consequences of warming for Arctic tundra plant functional group richness and ecosystem productivity, the impact of increasing nutrient availability on Arctic plant functional and phylogenetic diversity is relatively unknown. In addition, though the relationship between climate and the distribution and abundance of species through space and time is well established, how climate-related environmental change affects multiple dimensions of diversity simultaneously is understudied. Given our findings, it is important to more comprehensively quantify biodiversity and shifts in multiple dimensions of diversity with environmental change, as solely evaluating taxonomic or functional group diversity may underestimate the long-term responses of biological communities to warming-related nutrient enrichment and the ability of these communities to support multiple ecosystem properties and processes through time.

Acknowledgements

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Figures

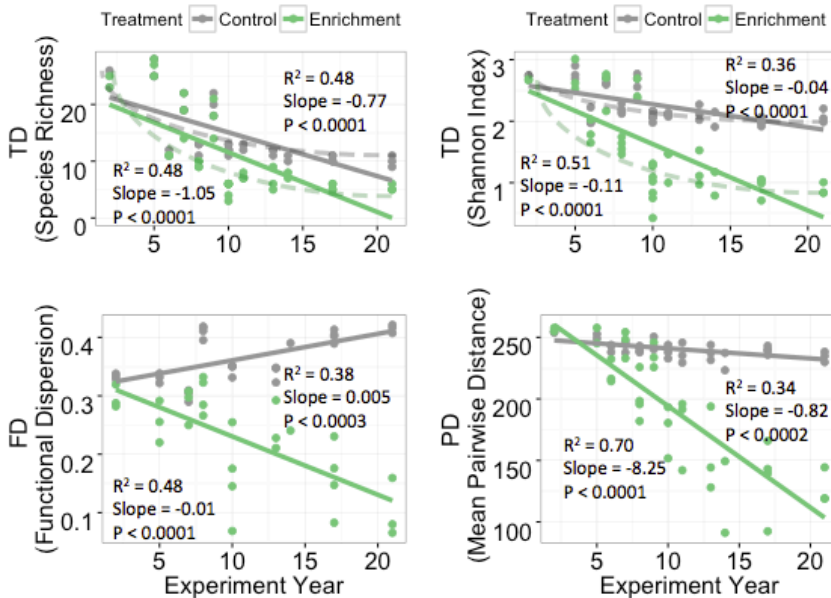


Figure 3.1 1

Figure 1. Taxonomic, functional and phylogenetic diversity (i.e., TD, FD, PD) over time for control and nutrient addition plots at the Arctic long-term research (ARC LTER) site. Taxonomic diversity was quantified by both species richness (a) and an abundance-weighted measure that prioritizes evenness (Shannon Index) in a community (b), FD was measured using functional dispersion (F_{Dis}), the mean distance of species from the centroid of all species, weighted by abundance (c), and PD was measured using an abundance-weighted metric, mean pairwise distance (MPD), that captures evolutionary relationships in a community (d). For all plots, control plots are in grey and nutrient addition plots are in green, points represent a plot-level average in a given year and in all panels, solid lines represent linear regressions and dashed lines represent best fit non-linear curves (a & b).

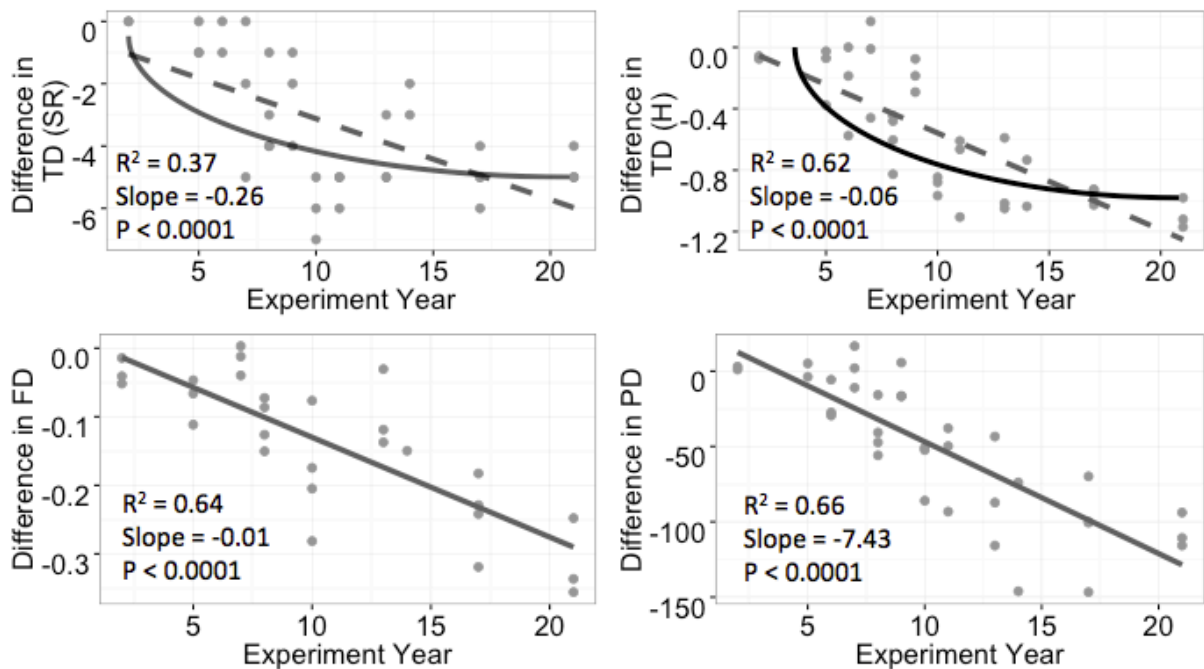


Figure 3.2 1

Figure 2. Relationships between the difference in diversity between control plots and nutrient addition plots through time. Points represent the value for a given nutrient addition plot minus the diversity value at a corresponding control plot, and all panels include a solid linear regression line. Fit statistics are not shown for plots (a,b) with no significant linear relationship, but are shown for those with significant relationships (c,d). Dashed linear regression lines show where best fit curve is non linear (exponentially decreasing trends shown in a & b).

CHAPTER 4 – Multiple dimensions of diversity drive ecosystem function in a natural system

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Introduction

As human-altered ecosystems now dominate our earth, great emphasis has been placed on understanding which species decrease, persist, or increase in these altered or newly created habitats and the consequences of declines in biodiversity for the functioning of ecosystems (Cardinale et al. 2012, Naeem et al. 2012). Biodiversity is now generally accepted to exhibit positive, saturating or decelerating relationships with ecosystem function (Tilman et al. 1997a, Tilman et al. 1997c, Chapin et al. 1998, Reich et al. 2004); however, the majority of existing work is experimental, and far less is understood about the causal relationship between biodiversity and ecosystem function across naturally assembled systems, the applicability of experimental findings to natural variation in diversity (Jiang et al. 2009), or the simultaneous influence of multiple dimensions of diversity (e.g., genetic, functional, phylogenetic, taxonomic, etc.) on measures of ecosystem function (Naeem et al. 2016b). Here, we show that simultaneous changes in multiple dimensions of plant diversity and whole ecosystem productivity are linked in Arctic tundra. We found that taxonomic diversity and functional diversity were significantly and positively related to whole ecosystem productivity, measured using net ecosystem CO₂ exchange and total aboveground biomass. Conversely, we found that functional evenness and dispersion – measures of functional diversity that quantify the distribution of plant traits within the trait space of a community – were significantly and negatively related to ecosystem productivity. While phylogenetic diversity was positively associated with ecosystem productivity, the relationship was not significant. Our results demonstrate how multiple dimensions of biodiversity simultaneously impact ecosystem function in complex ways in a natural system undergoing rapid environmental change. We suggest that integrating multiple dimensions of diversity allows for a more complete understanding of ecosystem function while simultaneously permitting inferences to be made into the underlying mechanisms of relationships between biodiversity and ecosystem

function in natural systems, an exciting potential avenue for future work integrating experimental and observational approaches.

Human alteration and simplification of ecosystems through habitat modification, the introduction of domestic and exotic species, and anthropogenic climate change, are leading to significant declines in biodiversity and the impairment of ecosystem functioning worldwide (Naeem et al. 2012). After over 20 years of research, an experimental and theoretical framework for exploring the connections between biodiversity and ecosystem function has produced widely accepted generalities about these relationships and dynamics (Tilman et al. 2014). This framework has focused on experiments in which species are randomly added or removed, providing a foundation for exploring the impacts of natural or human-induced variation in biodiversity in order to understand the impacts of alternate assembly and disassembly processes (Grime 1998, Wardle 1999, Huston et al. 2000, Jiang et al. 2009) on BEF relationships.

Results from previous experimental studies that have attempted to approximate real-world scenarios of biodiversity losses via non-random species removal, coupled with a limited number of observational studies, vary considerably, with some suggesting positive associations between diversity and function (Flombaum and Sala 2008, Zhu et al. 2016), and others finding no evidence of significant relationships (Weeks et al. 2016), or even negative associations (Wang et al. 2013). These conflicting results may suggest variation in relationships between biodiversity and ecosystem function across aspects of community composition and assembly (Weeks et al. 2016) or across ecosystem functions, but they may also reflect the challenges associated with replicating natural systems in experimental contexts. By exploring the connections between biodiversity and ecosystem function across observational gradients, it is possible to evaluate the applicability of experimentally derived findings for natural systems,

particularly those most affected by global environmental change.

While traditional experimental approaches have typically relied on manipulating taxonomic diversity (i.e. species richness and/or evenness), evaluating multiple dimensions of diversity in natural systems can both refine our understanding of the relationship between biodiversity and ecosystem function and has the potential to capture mechanisms underlying the relationship in a non-experimental context. Biodiversity is a complex, dynamic and often scale-dependent entity that cannot be readily reduced to a single value or dimension, and while biodiversity is well understood to be multidimensional, very few studies go beyond examining the connections between a single dimension of diversity and ecosystem function (Naeem et al. 2016b). Recent studies suggest that using functional diversity (FD) – quantifying the type, range and relative abundance of organismal traits in ecological communities – rather than taxonomic diversity (TD) can improve our ability to develop a mechanistic understanding of how and why biodiversity loss impacts ecosystem functioning and the services derived from these functions (Petchey et al. 2004, de Bello et al. 2009). In addition, phylogenetic diversity (PD) may explain significantly more variation in primary productivity than TD or FD (Flynn et al. 2011) because measured functional traits may be correlated with other important traits not included in FD metrics, but are often evolutionarily conserved and thus correlated. However, PD does not always reflect unmeasured traits that influence ecosystem properties or processes, as any additional information provided by PD may reflect ecologically irrelevant trait variation (Cadotte 2006, Cadotte et al. 2008, Losos 2008, Best et al. 2013). *A priori* determination of which dimension of diversity will be most relevant is not yet feasible, and may never be appropriate, making comprehensive multidimensional methods an important strategy for understanding the relationship between biodiversity and ecosystem function and the mechanisms thought to

underlie diversity's influence on ecosystem function (Wright et al. 2006, Carroll et al. 2011). In addition, examining multiple dimensions of diversity – combining an understanding of TD, FD and PD – will help better predict ecosystem consequences of realistic species losses due to global change.

Here, we examine the link between natural variation in multiple dimensions of plant diversity and ecosystem function in Arctic tundra, an ecosystem undergoing rapid warming, but relatively free of the effects of other global change drivers, such as habitat degradation, invasive species, and N deposition. We used structural equation modeling (SEM) to examine how multiple dimensions of plant diversity (FD, PD, and TD), and the distribution of functional traits through trait space, are related to two measures of ecosystem function associated with whole ecosystem C dynamics – aboveground biomass (AGB) and net ecosystem exchange (NEE). We test for the first time in a natural system whether or not different dimensions of diversity are positively associated with ecosystem function, owing to niche complementarity (a positive association between the evenness or dispersion of functional traits and ecosystem function) or selection effects.

Methods

Site description

All field sampling for this study took place during peak growing season (i.e., the period of peak tundra greenness) near the Arctic Long Term Ecological Research (ARC LTER) site and Toolik Field Station (TFS) located at Toolik Lake in the northern foothills of the Brooks Range, Alaska (68°38'N and 149°43'W, 760 m a.s.l.). All study plots are situated on moist acidic

tundra with soils comprised of 30–55 cm of a peaty organic and silty mineral layer, atop continuous permafrost. The growing season at the ARC LTER and TFS site spans 10-12 weeks, beginning in early to mid-June, with an average growing season temperature of 10°C. The period of peak leaf out for low Arctic tundra plant communities, dominated by graminoids (grass and sedge species) and deciduous and evergreen shrubs, is approximately 30-35 days (Sweet et al. 2015). All sampling for this study took place during the period of peak tundra leaf out in 2013 and 2014.

Ecosystem CO₂ exchange and aboveground biomass

During the period of peak tundra leaf out or greenness (mid-July 2013 and 2014), changes in CO₂ concentration, photosynthetically active radiation (PAR) and air temperature were measured using a Li-Cor 7500 infrared gas analyzer (IRGA; Li-Cor, Lincoln, Nebraska, USA) at 35 randomly selected plots (diameter = 1m). The IRGA was affixed to a cylindrical, portable chamber with a polyvinyl chloride (PVC) frame and transparent plastic sheeting with internal fans to ensure adequate mixing of air and steady chamber temperatures, atop a separate PVC base fitted with a plastic skirt, sealed to the ground with two heavy chains. At each sampling location, we conducted flux measurements to permit calculation of net ecosystem exchange (NEE). Each measurement cycle began by lowering the chamber onto the base and sealing it. Once a consistent rate of CO₂ exchange was achieved, we began logging a two to three minute flux measurement in the light (for calculation of NEE) at a sampling frequency of 0.5 Hz. Once we completed a flux measurement, the flux chamber was opened and allowed to acclimate to ambient CO₂ concentrations (roughly 400 ppm) for 15-30 seconds before logging a second flux measurement. This cycle was repeated three times, yielding three flux

measurements in the light. The temperature in the chamber did not exceed 23.5 °C during any measurement. For each study plot, we averaged the three fluxes made in the light and we calculated NEE ($\mu\text{mol m}^{-2} \text{s}^{-1}$) using Eqn 1 to quantify the continuous exchange of CO_2 between the atmosphere, vegetation and soil.

$$\text{NEE} = (\rho * V * (dC/dt)/A) \quad (1)$$

In Eqn 1, ρ is the air density (mol air m^{-3}), defined as P/RT , where P is the average pressure (Pa), R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ air K}^{-1}$), and T (K) is the mean temperature. V is the chamber volume (m^3), dC/dt is the slope of the chamber CO_2 concentration against time ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air s}^{-1}$), and A is the surface area of the ground (m^2) within the chamber. Prior to calculating NEE, we converted CO_2 density into a dry mole fraction. Positive NEE values indicate fluxes from the atmosphere to the ecosystem and negative values indicate fluxes to the atmosphere from the ecosystem. Upon completion of CO_2 flux sampling, we harvested the aboveground biomass in three randomly selected subplots ($r=10\text{cm}$) in each plot, and sorted vascular plant biomass to species. After biomass harvests were sorted by species, we dried them at 60 °C for at least 48 hours or until weight measurements stabilized.

Trait data

For roughly 70% of the species found across all study plots, spanning rare and common species, we collected six leaf functional traits that are thought to be mechanistically linked to ecosystem processes related to C cycling and storage: specific leaf area (SLA), leaf carbon concentration (%C), leaf nitrogen concentration (%N), light saturated rate of net CO_2 assimilation (A_{max}), maximum respiration in the dark (R_{dark}), and integrated water use efficiency (WUE, the intrinsic magnitude of C gain per unit water loss, calculated using a C-isotope

approach as photosynthetic enzymes discriminate against the heavier stable isotope ^{13}C relative to ^{12}C during photosynthesis (Hubick and Farquhar 1989). For all traits, except leaf-level gas exchange measures, which were measured on 3-5 individuals, we sampled 7-10 individuals in or near our study plots, and we dried leaf tissue at 60 °C for 72 hours. All traits were sampled following standard protocols outlined by Cornelissen et al. (2003).

Dimensions of biodiversity

We examined three dimensions of biodiversity: (1) TD, quantified by two metrics, (2) PD, quantified by two metrics, and (3) trait-based FD, quantified by three metrics. Conceptually, we considered trait-space volume to be more coherent with species-based metrics than trait-distribution-based metrics, a distinction important in some of the statistical analyses.

Taxonomic diversity was measured using species richness (S), the Simpson Index (D), and Shannon's Index (H) indices that reflect evenness and dominance of species within a community. All measures of taxonomic diversity were calculated using the *vegan* package (Okasen et al. 2015) in R v. 3.2.1 (R Core Team 2015).

Phylogenetic diversity (PD) was estimated using two indices: mean pair-wise distance (MPD) and Faith's phylogenetic diversity (FPD). These metrics were calculated using the *picante* package in R (Kembel et al. 2010) with phylogenetic relationships and branch lengths obtained from a locally stored phylogeny in *phylomatic* (Zanne et al. 2014), processed using *Phylomatic V3* (Webb et al. 2008) and the *ape* (Paradis et al. 2004) and *phytools* (Revell 2012) packages in R. MPD is the mean sum of the phylogenetic branch lengths between all species pairs within a community. FPD represents the sum of all branch lengths connecting all of the members of a community and is highly correlated with species richness.

Functional diversity (FD) was characterized using three metrics of FD: functional richness, functional evenness, and functional dispersion (Villegger et al. 2008), implemented in the R package FD (Laliberte and Legendre 2010). All FD metrics were calculated using standardized values of three functional traits: specific leaf area (SLA), leaf nitrogen content (%N) and the ratio of foliar photosynthesis to respiration (A/R). We selected these three traits from our total trait pool, as they are mechanistically linked to C cycling, and by reducing the number of traits it enabled us to maximize the number of communities included in our analyses, as multidimensional trait-space FD metrics require more species in a community than traits. Functional richness (F_{Ric}) was used to estimate the total volume of a multidimensional trait space defined by the functional traits of the species in a community (Villegger et al. 2008). Functional evenness and functional dispersion were used to characterize the distribution of species through trait space. Functional evenness quantifies the regularity with which species are distributed throughout a multidimensional functional space. If the species in a community are clustered within functional space, F_{Eve} will be low, and vice versa. Similarly, F_{Dis} describes the distribution of species throughout a multidimensional space, but captures the degree to which species are close to the center of the functional space or close to the edges of the functional space. Communities with a high proportion of species clustered near the center of functional space will have low F_{Dis} , while communities with high proportions of species near the edges of the space will have high F_{Dis} values.

Statistical analyses

We used structural equation modeling (SEM) to analyze the relationships between multiple dimensions of biodiversity, NEE and total aboveground biomass (AGB) as well as an

integrated (latent) measure of ecosystem carbon (C) dynamics that integrates NEE and AGB. SEM is a set of statistical techniques that allows for complex relationships between one or more independent variables and one or more dependent variables using a combination of regression and factor analysis methods, and is reviewed in Mitchell (1992), Hershberger (2003), and Grace et al. (2012). The influence of multiple dimensions of biodiversity on ecosystem function were modeled using SEM, implemented in the R package *lavaan* (Rosseel 2012). Following Weeks et al. (Weeks et al. 2016) we modeled the distribution of functional traits through trait space as a latent variable (here, “functional trait distribution”), and treated functional richness as a distinct exogenous variable. The latent functional trait distribution variable was measured using F_{Eve} and F_{Dis} , with a variance fixed to 1 and the loading on functional evenness constrained to 1. Faith’s PD, F_{Ric} and the Simpson Index (D) were considered exogenous variables. Ecosystem function (both as a latent variable and an exogenous variable, with the variance fixed to 1, depending on the model) was regressed onto functional trait distribution, F_{Ric} , FPD , and D . Finally, in order to explicitly explore the distribution of species through functional trait space, we ran a principal component analysis using the package *stats* in R. We ordinated species using all six functional traits to examine the distribution of species in functional trait space.

Results

We used two SEM structures, one in which ecosystem function was considered a latent variable, measured by net ecosystem exchange (NEE) and total aboveground biomass (AGB; Fig. 1), and one in which both NEE and AGB were modeled individually in separate SEMs (Fig. S1; *Methods*). When we treated ecosystem function as a latent variable, the best-supported model explained 60% of the variance in ecosystem function and was significantly better than a null model ($n = 33$, $\chi^2 = 59$, $df = 10$, $P < 0.0001$; Fig. 1). In addition, we found that functional diversity (FD) as measured by the functional richness of a community (F_{Ric} ; *Methods*) and

taxonomic diversity (TD) as measured by the Simpson Index (D) were significantly, positively related to ecosystem function (3.49 ± 1.72 , $z = 2.03$, $P < 0.05$; 1.3 ± 0.33 , $z = 3.91$, $P < 0.0001$; Fig. 1). Phylogenetic diversity (PD) as measured by Faith's PD was positively related to ecosystem function, but the relationship was not statistically significant. In addition, functional trait distribution was negatively related to ecosystem function (-2.79 ± 0.55 , $z = -5.04$, $P < 0.0001$; Fig. 1).

Next we decomposed ecosystem function into its measured components, NEE and AGB, and modeled them individually (Fig. S1; *Methods*). We found that the model was significantly better at explaining variation in NEE than a null model ($n = 33$, $\chi^2 = 54.6$, $df = 6$, $P < 0.0001$; Fig. S1a), and explained roughly 85% of the variance in NEE. For this model, only TD was positively related to NEE (1.36 ± 0.34 , $z = 4.01$, $P < 0.0001$; Fig. S1a) and functional trait distribution was significantly negatively associated with NEE (-3.03 ± 0.6 , $z = -5.1$, $P < 0.0001$; Fig. S1a). For AGB, the model is significantly better than a null model ($n = 33$, $\chi^2 = 54.4$, $df = 6$, $P < 0.0001$; Fig. S1a), and explained roughly 35% of the variance in AGB. Functional trait distribution was the only variable – exogenous or latent – significantly (negatively) associated with AGB (-1.31 ± 0.5 , $z = -2.67$, $P < 0.001$; Fig. S1b).

We subsequently performed a principal components analysis (PCA) on the species present in at least 10% of plots using our five plant traits (LMA, %C, %N, A_{\max} , and R_D). In the PCA, 49% of the variation in the data could be explained by the first axis and 23% by the second axis (Fig. 3; Table 1). The traits with the highest loadings on PC1 were LMA and A_{\max} , and the second axis was primarily determined by %N (Table 2). The species included in this analysis tended to group loosely by functional type (e.g., deciduous shrubs, evergreen shrubs, forbs and grasses/sedges; Fig. 3).

Discussion

Overall, we found evidence of the positive effects of biodiversity on ecosystem functioning across an observational gradient in Arctic tundra. While we found a positive relationship between FD (functional richness, F_{Ric}), PD (Faith's PD, FPD), TD (Simpson Index, D) and whole ecosystem C cycling and storage, only FD and TD were significantly, positively related to ecosystem function, demonstrating that in natural systems different dimensions of diversity may be differentially related to ecosystem function. This is in contrast to most experimental findings, where studies examining more than one dimension of diversity tend to find consistent relationships, regardless of the dimension of diversity examined (Flynn et al. 2011). Interestingly, our finding that both the magnitude and the direction of the relationship between biodiversity and function depend on the dimension of diversity considered, is less consistent with experimental studies within the same taxonomic group (plants) than it is with other studies that differ in terms of taxa, but also examine connections between biodiversity and ecosystem properties across a natural system (Weeks et al. 2016). This could reflect an important difference between experimental and natural contexts – for example, differences between diversity (Jiang et al. 2009) or broader assembly processes (Weeks et al. 2016) – that may not be captured by studies examining univariate diversity. This is important both for understanding relationships between diversity and function, but also because it may reflect differences in mechanisms underlying these relationships across natural and experimental systems.

The most widely supported mechanisms hypothesized to underlie the positive, saturating relationship between biodiversity and ecosystem functioning observed in theoretical and experimental studies are niche complementarity (Tilman 1997, Tilman et al. 1997b), selection effects (Tilman et al. 1997b), or a combination of the two (Hooper et al. 2005, Chiang et al. 2016, Grace et al. 2016). We hypothesize that increases in trait volume, or functional richness

(F_{Ric}), represent new, potentially complementary traits entering the community, which might be consistent with increasing niche complementarity and/or a greater opportunity for dominance to result in selection effects. In addition, we hypothesize that decreases in functional trait distribution within trait space may reflect greater redundancy among species because more evenly and widely dispersed traits reflect more comprehensive trait-space coverage. Increasing redundancy among species may both lessen the likelihood of niche complementarity or, where dominance occurs, selection effects to positively influence ecosystem function and ultimately constrain possible community responses to future dimensions of global change (e.g., species invasions, pathogen introduction, land-use change). Though, in the absence of experimentation, it is not possible to definitively test these hypotheses, we suggest that by unifying observational work such as ours, with subsequent experimental approaches – both by returning to observational sites and conducting experimental work and by evaluating multiple dimensions of diversity within experimental studies that have focused on taxonomic diversity alone – it may be possible to establish whether or not the relationships across different dimensions of diversity are as informative as we posit here. Such a finding would allow for widespread evaluation of the mechanisms underlying relationships between biodiversity and function in natural systems.

Unlike experimental studies in which covarying factors are controlled to maximize the detection of diversity effects, multiple covarying factors may enhance, dilute, confound, or prevent the detection of diversity effects. Here, we demonstrate that diversity effects in Arctic tundra, an ecosystem experiencing rapid environmental change, may experience significant changes in C cycling and storage depending on the nature of the changes in biodiversity, not just changes in hydrology and nutrients as many studies have argued. More broadly, our findings have important implications for assumptions about the role of biodiversity in mediating

ecosystem function across naturally assembled systems and highlight the functional importance of biodiversity in environments experiencing rapid environmental change. In addition, our findings demonstrate the importance of simultaneously examining multiple dimensions of diversity, as ecosystem function may be differentially governed by certain aspects of biological diversity not captured by unidimensional metrics, allowing for inferences to be made about the mechanisms underlying the relationship between biodiversity and ecosystem function.

Acknowledgements

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Tables

Table 4.1 1

Table 1. Plant Trait Principal Component Analysis

Principal Component Axis	PC1	PC2	PC3	PC4
Proportion of Variance Explained	0.49	0.23	0.16	0.11
Cumulative Variance Explained	0.49	0.72	0.89	1.00

Table 4.2 1

Table 2. Plant Trait Principal Component Analysis Loadings

Trait	PC1	PC2	PC3	PC4
N	0.46	-0.58	0.60	-0.31
A	0.56	0.34	0.26	0.70
LMA	-0.47	0.46	0.74	-0.12
R	0.50	0.58	-0.15	-0.62

Figures

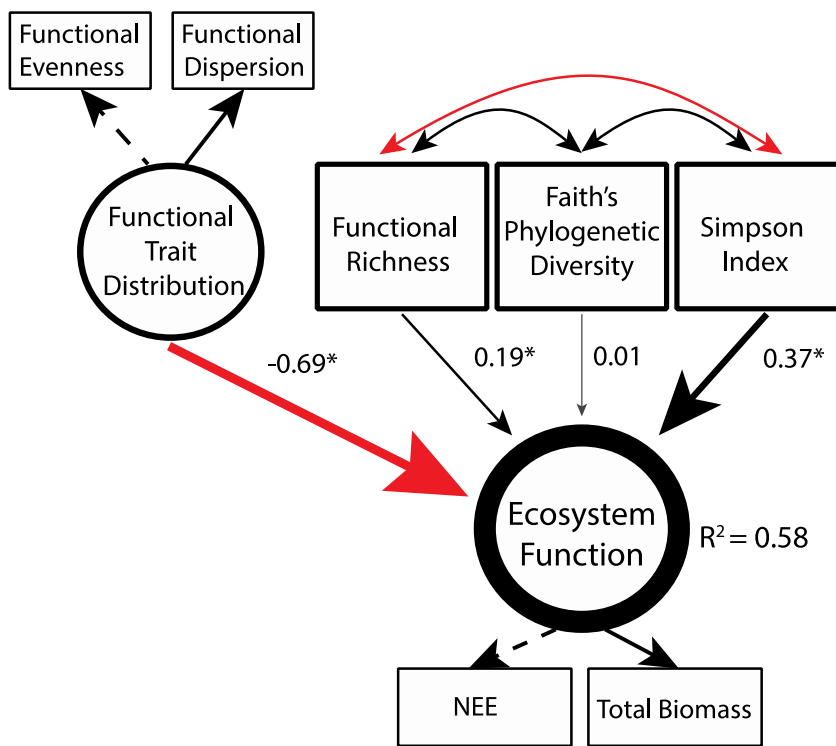


Figure 4.1 1

Figure 1. Structural equation model of the relationship between the distribution of functional traits, functional richness, Faith's phylogenetic diversity and taxonomic diversity (Simpson Index) and ecosystem function (a latent variable in the model measured by net ecosystem exchange [NEE] and total aboveground biomass) shows a positive relationship between functional richness and the Simpson Index and function. In addition, there is a negative relationship between the distribution of functional traits within trait space and ecosystem function. Parameter estimates are standardized, and the paths are scaled to reflect effect size. Significant relationships are denoted with asterisks. The fixed loading of the distribution of traits on functional evenness and the fixed loading of NEE on ecosystem function are shown as dashed lines. Here, the four dimensions of diversity explain roughly 60% of the variance in ecosystem function.

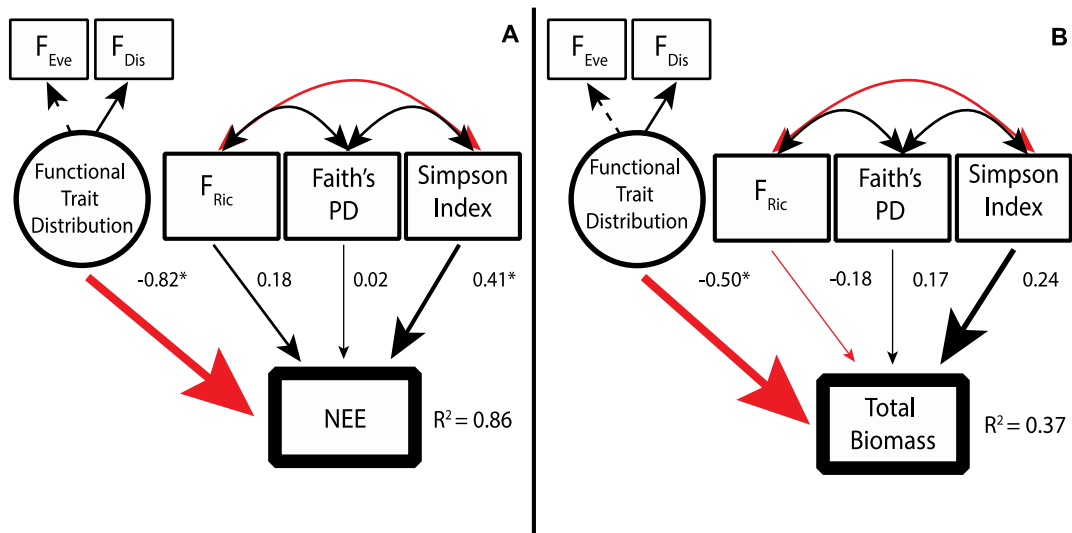


Figure 4.2 1

Figure 2. Structural equation models of the relationship between the distribution of functional traits, functional richness, Faith's phylogenetic diversity, the Simpson Index (taxonomic diversity) and (a) net ecosystem exchange (NEE) or (b) total aboveground biomass. (a) There is a significant and positive relationship between the Simpson Index and NEE and a significant negative relationship between functional trait distribution and NEE. All other relationships are positive, but not significant. Here, the four dimensions of diversity explain 86% of the variance in ecosystem function. (b) A significant negative relationship between the distribution of functional traits within trait space and total biomass. Parameter estimates are standardized, and paths are scaled to reflect effect size. Significant relationships are denoted with asterisks. The fixed loading of the distribution of traits on functional evenness and the fixed loading of NEE on ecosystem function are shown as dashed lines. Here, the four dimensions of diversity explain roughly 40% of the variance in ecosystem function.

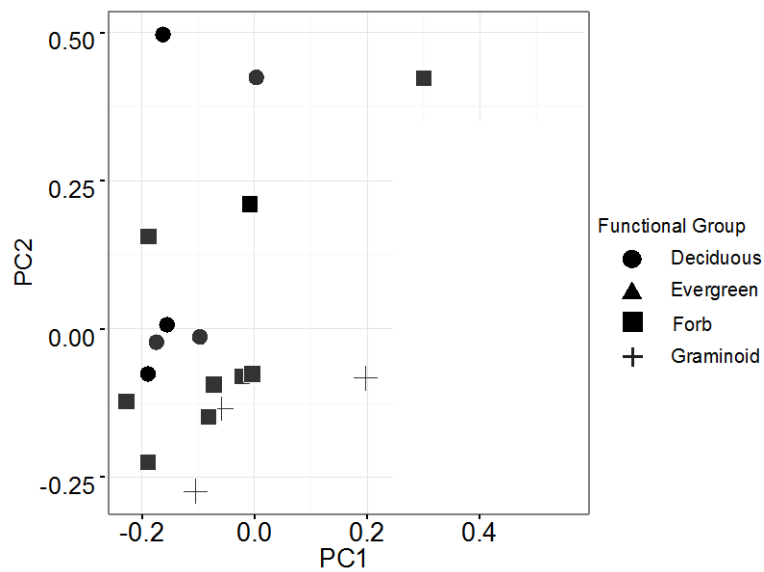


Figure 4.3 1

Figure 3. Principal component analysis of the species present in at least 10% of study plots by our six plant traits (i.e., SLA, %C, %N, A_{\max} , R_D and WUE). We found that 49% of the variation in the data could be explained by the first axis and 23% by the second axis, and the main traits driving the first axis were SLA and A_{\max} , in opposite directions, and the second axis was primarily determined by %N. The species included in this analysis tended to loosely group by functional type (e.g., deciduous shrubs, evergreen shrubs, forbs and grasses/sedges).

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