

Stoichiometric homeostasis: a test to predict tundra vascular plant species and community-level responses to climate change¹

Qian Gu, Tara J. Zamin, and Paul Grogan

Abstract: Climate change is having profound influences on Arctic tundra plant composition, community dynamics, and ecosystem processes. Stoichiometric homeostasis (H), the degree to which a plant maintains its internal nutrient concentrations independent of nutrient variations in its environment, may be a useful approach to predict the impacts of these influences. In this case study, we used fertilization manipulation data to calculate homeostasis indices based on nitrogen (H_N), phosphorus (H_P), and nitrogen to phosphorus ratios ($H_{N:P}$) of aboveground tissues for seven common tundra vascular species belonging to three growth forms. We then analyzed species H relationships with dominance, spatial stability, and responsiveness to various experimental manipulations. Each of the H indices was correlated amongst tissue types within each species and was generally highest in ericoid mycorrhizal host species and lowest in the ectomycorrhizal birch. Species H_P and $H_{N:P}$ were consistently positively correlated with aboveground biomass within the controls and across all manipulations. Furthermore, these same species were spatially stable across experimentally warmed field plots. Stoichiometric homeostasis theory has been successful in predicting grassland community dynamics. This first test of its applicability across a variety of Arctic plant growth forms highlights its considerable potential in predicting tundra plant community structure and responses to environmental change.

Key words: Arctic tundra, nitrogen, phosphorus, species dominance, spatial stability.

Résumé : Le changement climatique a des effets considérables sur la composition des plantes de la toundra arctique, la dynamique de communauté et les processus d'écosystème. L'homéostasie stœchiométrique (H), le degré auquel une plante maintient ses concentrations de nutriments internes indépendamment des variations de nutriments dans son environnement, peut être une approche utile pour prédire les impacts de ces effets. Dans cette étude de cas, nous avons utilisé des données de manipulation de fertilisation afin de calculer les indices d'homéostasie en fonction de l'azote (H_N), du phosphore (H_P) et des rapports azote à phosphore ($H_{N:P}$) de tissus épigés, et ce, pour sept espèces vasculaires de toundra communes appartenant à trois formes de croissance. Nous avons alors analysé les relations d' H des espèces en rapport avec la dominance, la stabilité spatiale et la réactivité à diverses manipulations expérimentales. Chacun des indices d' H a été corrélé parmi les types de tissus propres à chaque espèce et était généralement le plus élevé chez les espèces hôtes éricoïdes mycorrhiziennes et le plus bas chez le bouleau ectomycorhizien. L' H_P et l' $H_{N:P}$ des espèces étaient systématiquement positivement corrélés avec la biomasse épigée entre les contrôles et pour toutes les manipulations. En outre, ces mêmes espèces étaient stables spatialement sur l'ensemble des parcelles de terrain réchauffées à titre expérimental. La théorie d'homéostasie stœchiométrique a eu du succès à prédire la dynamique des communautés de prairie.

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Ce premier test de son applicabilité sur l'ensemble d'une variété de formes de croissance de plantes arctiques met en évidence son potentiel considérable dans la prédiction de la structure de communauté de plantes de toundra et des réactions au changement environnemental

Mots-clés : toundra arctique, azote, phosphore, dominance d'espèce, stabilité spatiale.

Introduction

Ecosystems across the globe are undergoing stresses in recent decades due to climate change, atmospheric nitrogen deposition, invasive species, and land use change (Elser et al. 2010). To make useful predictions of future ecosystem responses, it is critical to understand the mechanisms that underpin ecosystem structure, functioning, and stability. Plant communities and their functional characteristics are key components of terrestrial ecosystems. Among various plant traits, strategies for resource use link the functioning of individual species to major environmental factors, making them increasingly useful to ecosystem ecologists (Elser et al. 2010). A new concept — ecological stoichiometry — has been developing in recent years to explicitly and mechanistically characterize that link (Sturner and Elser 2002; Elser et al. 2010).

Ecological stoichiometry is a conceptual framework that considers how the balance of energy (C) and elements (particularly N and P) affects and is affected by organisms in the environment. Stoichiometric homeostasis is the degree to which an organism maintains relatively constant concentrations or ratios of elements within its tissues despite variation in the relative availabilities of these elements in its environment or food supply (Sturner and Elser 2002; Elser et al. 2010; Yu et al. 2010, 2015). Stoichiometric homeostasis theory has been investigated and validated primarily in marine and freshwater ecosystems (Frost et al. 2002, 2005; Demars and Edwards 2007). However, our understanding of its regulation and role in affecting organismal and ecosystem processes in terrestrial ecosystems is very limited. Only three studies have attempted to relate stoichiometric homeostasis with vascular species and (or) terrestrial ecosystem stability, dominance, and production, and all of those were conducted in temperate grasslands (Yu et al. 2010, 2015; Dijkstra et al. 2012). For example, species H_N and $H_{N:P}$ were positively correlated with species dominance and with temporal stability (i.e., biomass consistency over a specified time period (Lehman and Tilman 2000) - see "Materials and methods" section for calculation) on both short-term and long-term temporal scales and across a large spatial scale in Inner Mongolian grasslands (Yu et al. 2010). Furthermore, community H_N was also positively correlated with community production and stability in most instances (Yu et al. 2010). The most dominant species in a C_3 grassland in Wyoming, USA, had the highest $H_{N:P}$, but in that case, there was no general correlation between species $H_{N:P}$ and species dominance (Dijkstra et al. 2012). However, consistent with the Inner Mongolia study (Yu et al. 2010), species H_N was positively correlated with species dominance and with stability on long-term (25 year) temporal scale in a central US grassland (Yu et al. 2015). Moreover, species H_N was also predictive of species responsiveness to experimental changes in soil water availability, with higher H_N species being less responsive (i.e., more resilient) to extreme drought, increased rainfall variability, and chronic increases in rainfall (Yu et al. 2015). These studies indicate that stoichiometric homeostasis can be usefully applied to predicting herbaceous species dynamics in grassland ecosystems to varying degrees. However, whether these capacities of stoichiometric homeostasis apply to other growth forms or ecosystem-types remains unknown.

Arctic tundra terrestrial ecosystems are globally important because they occupy a large area (CAVM 2003), their structure and functioning are sensitive to subtle changes in climate (ACIA 2004), and many of the impacts of rising temperatures such as shrub and tree expansion, soil warming, and permafrost thaw could have large effects on atmospheric greenhouse gas concentrations (Chapin et al. 2000). Climate change due to anthropogenic fossil fuel emissions

has resulted in rising Arctic air temperatures over the past 30 years (Serreze and Francis 2006), and since models consistently predict that temperatures will continue to rise most rapidly in high latitudes (Johannessen et al. 2004; IPCC 2016), there is a growing urgency to better understand how and why tundra vegetation will respond to warming. To the best of our knowledge, no studies have as yet quantified stoichiometric homeostasis values for Arctic vascular plant species, let alone investigated if stoichiometric homeostasis could be useful in predicting tundra species and community responses to environmental change.

According to Sterner and Elser (2002), the stoichiometric homeostatic regulation coefficient (termed as “ H ” hereafter) can be calculated through the following equation: $y = cx^{1/H}$, where y is the plant N or P concentration or N:P ratio, x is the concentration of available N or P or N:P ratio in the soil, and c is a constant. The “ H ” value is calculated as the reciprocal of the exponent value of the exponential function (H can range from 1 (no homeostasis) toward infinity (high homeostasis)). Species with higher H values are more homeostatic (i.e. they tend to maintain relatively constant tissue nutrient concentrations across a wide range in soil nutrient availability).

Challenges of applying this equation (termed as “ H model” hereafter) in tundra ecosystems are (1) for y , which part of plant tissue is most representative of overall homeostasis for a given species and (2) for x , how to comprehensively assess the nutrients that are biologically available to plants from tundra soils. Although previous studies have verified the applicability of the H model in US and Asian temperate grasslands using concentrations of leaf nutrients to represent “ y ” and concentrations of soil inorganic N or phosphate to represent “ x ” (Yu et al. 2010, 2015), these variables may not be as appropriate for tundra ecosystems. Unlike temperate grasslands where the dominant plants are all herbaceous species whose biomass is strongly dominated by leaves and where soil available N and P pools are dominated by inorganic compounds (e.g., ammonium and nitrate for N, phosphate for P) (Yu et al. 2010, 2015; Dijkstra et al. 2012), tundra ecosystems contain a wide range of plant growth forms, and various chemical forms of critical soil nutrients can be taken up by Arctic plants (McKane et al. 2002; Turner 2008). For example, the N that is available to tundra vegetation is dominated by organic N (various amino acids), but there are also ammonium and nitrate forms, and individual plant species have accordingly diversified to favor these different chemical forms (McKane et al. 2002). For P, there are likewise various organic and inorganic forms in tundra soils (Turner 2008), but nevertheless, plant P accumulation for a wide range of tundra species is closely correlated with soil phosphate (Kielland and Chapin 1994).

The vegetation of low Arctic tundra includes evergreen shrubs, deciduous shrubs, graminoids, and forbs (Chapin et al. 1980) that all differ in their growth rate, stature, storage capacity, organ longevity, and nutrient allocation patterns among tissues (Chapin and Shaver 1988, 1989). These differences may lead to growth-form-specific H values across different plant species and age- and tissue-specific H values for a given species. For herbaceous tundra species, leaves are the dominant aboveground tissue, and therefore, just as for grassland species (Yu et al. 2010), foliar nutrient concentrations may be appropriate for calculating plant species H . However, shrub species have considerable woody tissue as well as leaves, and a substantial proportion of the wood and leaves may have been formed in previous years, altogether resulting in more complex plant internal nutrient allocation patterns. Therefore, H values based on leaf nutrient concentrations may be less appropriate and other aboveground tissues may produce a better estimation of overall plant H (Elser et al. 2010).

This case study is the first to investigate the applicability of stoichiometric homeostasis to Arctic tundra vascular plant species and to link it with species and community dynamics in response to altered environmental conditions. We used plant shoot biomass and nutrient pool data from a set of long-term experimental manipulations in low Arctic tundra to address the following three questions: (1) What are the age-specific, tissue-specific, and total

aboveground shoot H (termed as “shoot H ” hereafter) values for a given species and do they correlate with each other? (2) Are species H values predictive of species biomass dominance, biomass spatial stability, and biomass responsiveness to experimentally induced changes in climate and soil nutrient availability? (3) Is community H predictive of community biomass?

Materials and methods

Field sampling

Our study used field data from experimental treatment plots that had been running for 8 years (greenhouse warming, low N addition, high N addition, and high P addition; $n = 5$ of each) in a mesic birch hummock tundra ecosystem near the Tundra Ecosystem Research Station at Daring Lake, Northwest Territories, Canada ($64^{\circ}52'N$, $111^{\circ}33'W$). Average annual air temperature in this area is $-9^{\circ}C$, with mean diel (i.e., 24 h period) temperatures ranging from $-40^{\circ}C$ in January to $20^{\circ}C$ in July. Annual precipitation ranges from 200 to 300 mm, with ~ 140 mm falling as rain in the summer and ~ 30 – 40 cm of snow accumulating during the winter in low-lying flat areas (all data are 1996–2013 averages; Bob Reid and Shawne Kokelj, unpublished).

We focused on the seven major vascular plant species in this ecosystem. These species belong to three growth forms: deciduous shrubs (*Betula glandulosa* Michx. and *Vaccinium uliginosum*), evergreen shrubs (*Rhododendron subarcticum* Harmaja (formerly *Ledum decumbens* (Aiton) Lodd. Ex Steud.), *Vaccinium vitis-idaea* L., and *Andromeda polifolia* L.), and herbaceous species (*Eriophorum vaginatum* and *Rubus chamaemorus*). Besides vascular species, there is also a well-developed moss and lichen layer, which constituted $\sim 44\%$ of the aboveground live plant biomass (see Nobrega and Grogan (2008) and Zamin et al. (2014) for more details).

In the N addition experiments, NH_4NO_3 was added at three levels (0, 1, and $10\text{ g N m}^{-2}\text{ year}^{-1}$; $n = 5$), while in the P addition treatment, P was added (as 45% P_2O_5) at two levels (0 and $5\text{ g P m}^{-2}\text{ year}^{-1}$; $n = 5$). Fertilizers were applied once yearly, generally in late August, since 2004. The experimental warming was achieved with A-frame greenhouses ($1.8\text{ m} \times 4.7\text{ m}$; $n = 5$) covered with heavy polyethylene film ($150\text{ }\mu\text{m}$) that was generally put on by late June and taken off by late August each year since 2004 (Zamin et al. 2014). Aboveground plants from one $40\text{ cm} \times 40\text{ cm}$ quadrat from each plot were harvested by cutting horizontally just below the green–brown moss transition in mid-August 2011 (Zamin et al. 2014). Briefly, all living vascular plants were first sorted to species and then sorted to age- and tissue-specific levels. For the deciduous shrubs, these age- and tissue-level differentiations included leaves, new stems, and old stems, for the evergreens, these included new leaves, old leaves, new stems, and old stems, for the graminoid (*E. vaginatum*), these included leaf sheaths and leaf blades, and for the forb (*R. chamaemorus*), all aboveground biomass was classified as “shoot”. Sorted samples were oven-dried at $40^{\circ}C$, weighed, and then ground for analysis of total N and total P. Surface organic soil samples ($5\text{ cm} \times 5\text{ cm}$ to 10 cm depth) were collected from the same plots during the mid-growing season (12–13 July 2011). See Zamin et al. (2014) for full details about the plant and soil sampling procedures.

Analysis of elements

N and P concentrations (% of dry mass) of plant samples were analyzed on the age- and tissue-differentiated tissue components by combustion and gaseous N detection (Elementar, Hanau, Germany) and inductively coupled plasma – atomic P spectrometry (ICP-AES) (Varian Vista AX, Palo Alto, California), respectively.

Soil ammonium-N (NH_4^+ -N), nitrate-N (NO_3^- -N), total dissolved N (TDN), and phosphate-P (PO_4^{3-} -P) were measured by extracting soil samples (10 g fresh weight) in either 0.5 M K_2SO_4 (for NH_4^+ -N and TDN analysis), Type I H_2O (NCCLS Type I resistivity: 10–18 $M\Omega\text{-cm}$; for NO_3^- -N analysis) or 0.5 M $NaHCO_3$ (pH 8.5; for PO_4^{3-} -P analysis). NH_4^+ -N, NO_3^- -N, and

$\text{PO}_4^{3-}\text{-P}$ concentrations in the extracts were determined colorimetrically using automated flow analysis (Bran-Leubbe Autoanalyzer III, Norderstadt, Germany) and the indophenol, sulphanilamide (Mulvaney et al. 1996), and molybdate – ascorbic acid methods (Kuo 1996), respectively. TDN concentrations in the extracts were determined by chemiluminescence (TOC-TN autoanalyzer, Shimadzu, Kyoto, Japan). N and P concentrations in the extracts were corrected for dilution associated with initial soil moisture in each individual sample and then calculated on a dry soil weight basis ($\mu\text{g N/P g}^{-1}$ dry weight soil). Soil water contents were determined by oven-drying soil samples at 65 °C to constant weight.

Data analysis and statistics

We addressed our first question (i.e., the applicability of the H model) on the seven vascular species and on the separate organs of these species using plant and soil samples from the control and N and P fertilization plots. We addressed our second and third questions using plant and soil samples from control plots and all four sets of experimental treatment plots. To investigate the applicability of the H model to the tundra species, we tested both the H model (power regression) and a linear regression model ($y = ax + b$) on plant nutrient concentrations/ratios and corresponding soil nutrient concentrations/ratios data.

Species dominance was assessed as the percent aboveground biomass for each species and community biomass was summed as the total vascular plant species aboveground biomass. Species spatial stability was defined in an analogous way to Lehman and Tilman's (2000) definition of temporal stability (i.e., the mean abundance divided by the standard deviation of that abundance data over time). Consequently, a variable with relatively low variation over time (or across space) would have a higher level of temporal (or spatial) stability. Specifically, in this study, species spatial stability (SS) equals species mean aboveground biomass (μ) divided by its standard deviation (σ) across the replicate plots in each treatment ($n = 5$): $\text{SS} = \mu/\sigma$. Species responsiveness to warming and fertilization manipulations was expressed as response ratio (RR), which was calculated by the equation of $\text{RR} = |\ln(x_m/x_c)|$, where x_m was the mean biomass for a species under a given treatment and x_c was the mean biomass for the species in the control plots (Yu et al. 2015). Community H for N, P, or N:P ratio was estimated as the sum of each species' percent aboveground biomass multiplied by its foliar H value for N, P, or N:P ratio, respectively (i.e., community $H = \sum_{i=1}^{n=7} \text{percent biomass of species } i \times H_i$) (Yu et al. 2010).

After calculating these indicators, their relationships with the corresponding H values were tested by Pearson's correlation when the assumption of normality was met; otherwise, Spearman's rank correlation was used. All statistical analyses were performed using R (version 3.2.3) (R Core Team 2015).

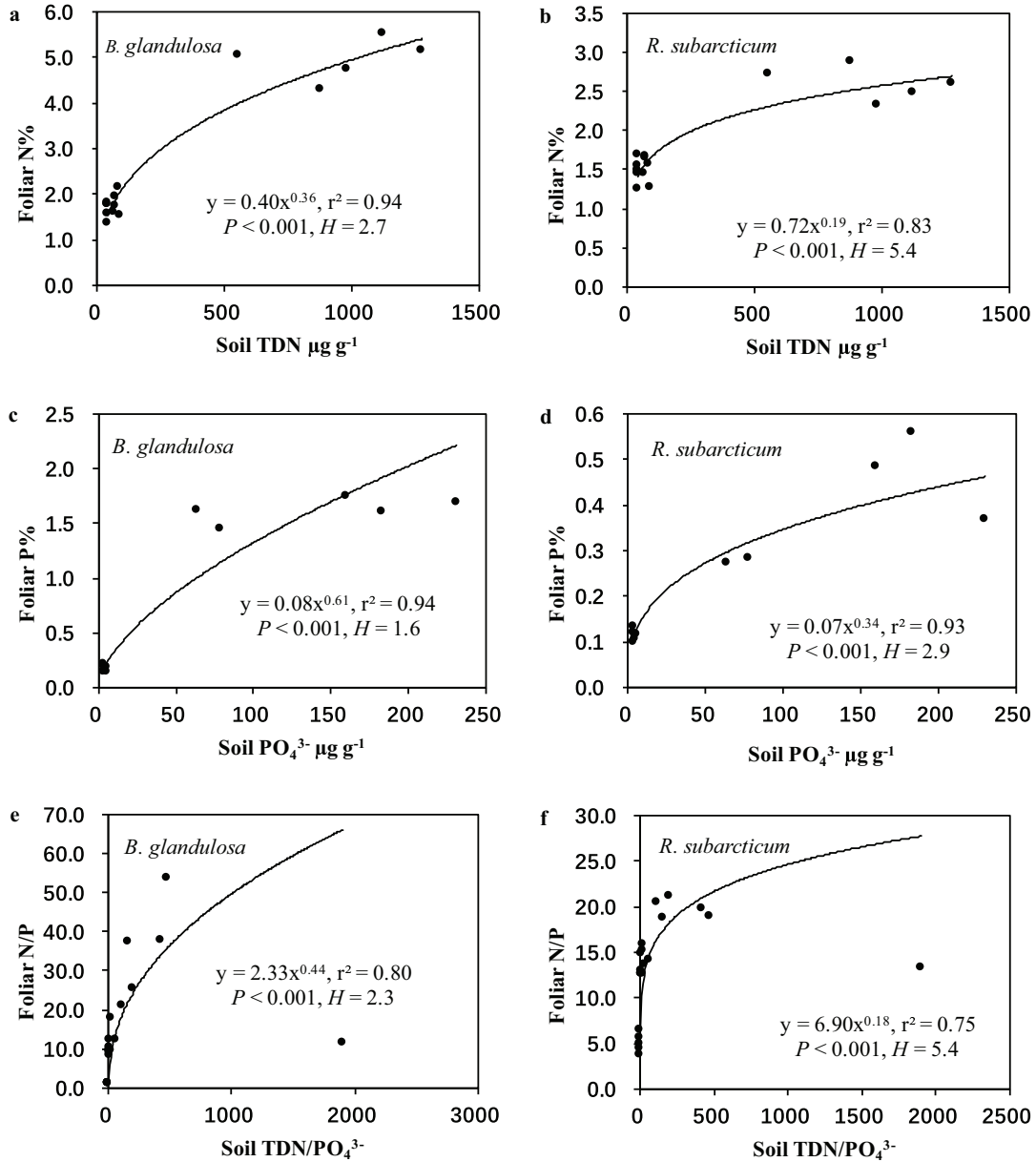
Results

Applicability of the H model to tundra species and to different soil nutrient forms

The H model was very effective in describing the relationships between plant shoot nutrient concentrations and soil nutrient pools across most of our tundra data set (Table 1; supplemental material Tables A1–A5²). For example, the H model fit for two species, *B. glandulosa*, the dominant deciduous shrub, and *R. subarcticum*, the dominant evergreen shrub, described 75%–94% of the variation between foliar N, P, or N:P and soil N, P, or N:P, respectively (Fig. 1). The only exception was H_N for the forb (*R. chamaemorus*), where the variability in its shoot N concentration in relation to soil available N concentration was not well described by the H model ($r^2 = 0.23$) (Table 1) because its shoot N concentrations decreased rather than increased in the high N addition treatment. However, the

²Supplementary material is available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/as-2016-0032>.

Fig. 1. Relationships between concentrations of (a and b) foliar N and soil TDN, (c and d) foliar P and soil PO_4^{3-} , and (e and f) foliar N:P and soil TDN: PO_4^{3-} for *B. glandulosa* and *R. subarcticum*, respectively, across control and N- and P-fertilized plots (see Table 1 legend for details). The “H” value is calculated as the reciprocal of the exponent value of the exponential function for each graph (H can range from 1 (no homeostasis) toward infinity (high homeostasis)).



associations of its shoot P concentration and N:P ratio with soil PO_4^{3-} concentration and N:P ratio were well described by the H model (Table A1). In terms of testing the mathematical nature of the stoichiometric linkages across the entire data set, relationships between plant species N or P concentration or N:P ratio and soil N or P concentration or N:P ratio were much better described by the H (power regression) model than by the linear regression model (Table A6).

Table 1. Foliar H (H_N represents $H_{(TDN)}$, H_P represents $H_{(PO_4^{3-})}$, and $H_{N:P}$ represents $H_{(TDN)/(PO_4^{3-})}$, respectively) and associated r^2 and P values for the seven principal vascular species in mesic birch hummock tundra at Daring Lake, NWT.

Species	Growth form	H_N	r^2	P	H_P	r^2	P	$H_{N:P}$	r^2	P
<i>Betula glandulosa</i>	Deciduous shrub	2.7	0.94	<0.001	1.6	0.94	<0.001	2.3	0.80	<0.001
<i>Vaccinium uliginosum</i>	Deciduous shrub	5.6	0.83	<0.001	1.6	0.91	<0.001	2.3	0.77	<0.001
<i>Vaccinium vitis-idaea</i>	Evergreen shrub	4.8	0.77	<0.001	2.5	0.96	<0.001	4.1	0.72	<0.001
<i>Rhododendron subarcticum</i>	Evergreen shrub	5.4	0.83	<0.001	2.9	0.93	<0.001	5.4	0.75	<0.001
<i>Andromeda polifolia</i>	Evergreen Shrub	7.1	0.54	0.002	2.3	0.95	<0.001	3.1	0.83	<0.001
<i>Eriophorum vaginatum</i>	Graminoid	7.1	0.59	0.001	3.0	0.80	0.001	3.5	0.71	<0.001
<i>Rubus chamaemorus</i>	Forb	ns*	0.23	0.069	2.0	0.95	<0.001	3.4	0.69	<0.001

Note: The calculation of H_N used 15 data points (three treatments (control, low N addition, and high N addition) with five replicates for each), the calculation of H_P used 10 data points (two treatments (control and high P addition) with five replicates for each), and the calculation of $H_{N:P}$ used 20 data points (four treatments (control, low N addition, high N addition, and high P addition) with five replicates for each).

*No H_N value has been included for *R. chamaemorus* because the stoichiometric model explained very little of the variability in the data.

Since tundra species are known to differ in their preferences for the various forms of N in Arctic soils (McKane et al. 2002), we used soil NH_4^+ , NO_3^- , and TDN to separately represent soil available N and accordingly calculated three H indexes to represent species' ability to exhibit stoichiometric homeostasis for N ($H_{(NH_4^+)}$, $H_{(NO_3^-)}$, and $H_{(TDN)}$) and for the N:P ratio ($H_{(NH_4^+/PO_4^{3-})}$, $H_{(NO_3^-/PO_4^{3-})}$, and $H_{(TDN/PO_4^{3-})}$). For a given species, H_N values calculated from the three soil N chemical forms differed substantially from each other. However, these differences were consistent among species (i.e., species with high $H_{(TDN)}$ also had high $H_{(NH_4^+)}$ and $H_{(NO_3^-)}$) and likewise for $H_{N:P}$ (Tables A1–A5). Furthermore, the correlative associations of plant N concentration and N:P ratio with soil N concentration and N:P ratio were best described (i.e., had generally higher and more consistent r^2 coefficients) by $H_{(TDN)}$ and $H_{(TDN/PO_4^{3-})}$ than $H_{(NH_4^+)}$ or $H_{(NO_3^-)}$ and $H_{(NH_4^+/PO_4^{3-})}$ or $H_{(NO_3^-/PO_4^{3-})}$, respectively (Tables A1–A5). Accordingly, $H_{(TDN)}$ and $H_{(TDN/PO_4^{3-})}$ are used to represent H_N and $H_{N:P}$, respectively, from here on in this study (Tables 1 and 2).

Patterns of H across species and among tissues for a given species

Species foliar H_P and $H_{N:P}$ values were positively correlated with each other ($r^2 = 0.66$, $P = 0.026$), whereas no significant relationships were observed between foliar H_N and H_P or H_N and $H_{N:P}$. Furthermore, the four ericaceous species (*A. polifolia*, *V. uliginosum*, *R. subarcticum*, and *V. vitis-idaea*) that form ericoid soil mycorrhizal associations (the “ErM species”) generally had higher H values than *B. glandulosa*, the only species that forms ectomycorrhizal fungal associations (the “EcM species”) (Tables 1 and 2). For the two herbaceous species, the graminoid *E. vaginatum* had higher H_P and $H_{N:P}$ than the forb *R. chamaemorus* (Table 1). Similar rankings of species H values were observed for current year's leaves, for old-growth tissues, and for whole shoots (Table 2; Tables A1–A5), and therefore, we used foliar H to represent species H from here on.

Relationships between species H and species dominance, spatial stability, and responsiveness to environmental change

Species H_P and $H_{N:P}$ were both positively correlated with species dominance across the control plots and within each of the experimental treatments (warming, low N addition, high N addition (H_P only), and high P addition) (Table 3; Figs. 2a and 2b), indicating that species with high P-based stoichiometric homeostasis consistently tended to be the most abundant. Likewise, H_P and $H_{N:P}$ were both also positively correlated with species stability — but only in the warming and low N addition treatments (Table 3; Figs. 2c and 2d), suggesting that stoichiometric homeostasis traits that conferred spatial consistency in relative abundance across replicate plots were only manifested in the more moderate experimental

Table 2. H_N , H_P , and $H_{N:P}$ values for new-growth and old-growth stems of the five woody species, for old-growth leaves of the three evergreen shrubs and sheaths of the graminoids, and for whole shoots of all seven vascular species in the mesic birch hummock tundra.

Species	New-growth stems			Old-growth stems			Old-growth leaves (evergreens) and sheaths (graminoid)			Total shoots		
	H_N	H_P	$H_{N:P}$	H_N	H_P	$H_{N:P}$	H_N	H_P	$H_{N:P}$	H_N	H_P	$H_{N:P}$
<i>B. glandulosa</i>	4.5	2.7	4.4	3.9	3.3	5.2				3.4	2.0	3.2
<i>V. uliginosum</i>	10.2	4.7	6.5	6.2	7.7	7.4				7.5	2.6	3.7
<i>V. vitis-idaea</i>	4.6	3.8	4.5	4.0	3.5	4.7	3.6	2.0	3.1	3.8	2.5	3.7
<i>R. subarcticum</i>	6.6	3.8	8.5	6.3	4.1	7.3	4.2	2.4	4.8	5.6	2.9	6.1
<i>A. polifolia</i>	7.8	NA	NA	3.9	3.2	3.9	5.4	1.7	2.4	5.1	2.3	3.1
<i>E. vaginatum</i>							3.5	3.3	3.4	5.2	3.0	3.4
<i>R. chamaemorus</i>										ns	2.0	3.4

Note: See Table 1 legend for details of the experimental treatments used in the data sets and Tables A1–A5 for the associated r^2 and P values for each H value.

manipulations that are nevertheless probably most realistic of future tundra environmental conditions. Species responsiveness to the experimental manipulations was significantly negatively correlated with species H_P and $H_{N:P}$ in the low N addition treatment only (Table 3), indicating that those species with high P-based stoichiometric homeostasis tended to be relatively unresponsive to low N additions. In contrast with H_P and $H_{N:P}$, species H_N was negatively correlated with species dominance across the control plots and within each of the experimental treatments (Table 3), and there were no significant correlations between species H_N and species stability or responsiveness.

Relationships between community H and community biomass

Consistent with the species-level results, community H_P and $H_{N:P}$ were positively related to each other ($r^2 = 0.99$, $P < 0.001$) but not to community H_N . Furthermore, aboveground biomass of the whole community was positively correlated with H_P and $H_{N:P}$ across the different experimental manipulations (Fig. 3).

Discussion

The stoichiometric H model is applicable for tundra vegetation

In this case study, we successfully applied the stoichiometric H model (Sterner and Elser 2002) to seven major tundra vascular species belonging to three growth forms using data obtained from 8 year field N and P addition experiments. Comparison of the goodness of fit of the plant and soil data between the H and linear regression models verified that tundra plant nutrient concentrations are not simply a direct reflection of soil nutrients supply (i.e., plants are not “simply what they root in” (Elser et al. 2010)). Instead, species clearly differ in the extent to which they control their tissue nutrient concentrations in response to changes in soil nutrient availability, and this regulation is the outcome of complex interactions among environments, genetics, and physiology (Elser et al. 2010). This is an important insight because plant growth is generally more strongly limited by soil nutrient availability in tundra than in the temperate grassland ecosystems (Schimel and Bennett 2004) where homeostasis has been previously studied (Yu et al. 2010, 2015; Dijkstra et al. 2012). Therefore, our study demonstrating that Arctic plant species differ in the extent of their stoichiometric control provides a new potential mechanism that may contribute to understanding current patterns in tundra species distribution and abundance as well as their potential responses to environmental changes.

Table 3. Relationships between species H (H_N , H_P , and $H_{N:P}$) and species dominance in control, warming, low N addition, high N addition, and high P addition treatments, between species H_P and $H_{N:P}$ and species spatial stability (shoot biomass consistency across replicate plots) in warming and low N addition treatments (relationships in controls and the other treatments were not significant; data not shown), and between species H_P and $H_{N:P}$ and species responsiveness in low N addition treatments (relationships in the controls and the other treatments were not significant; data not shown).

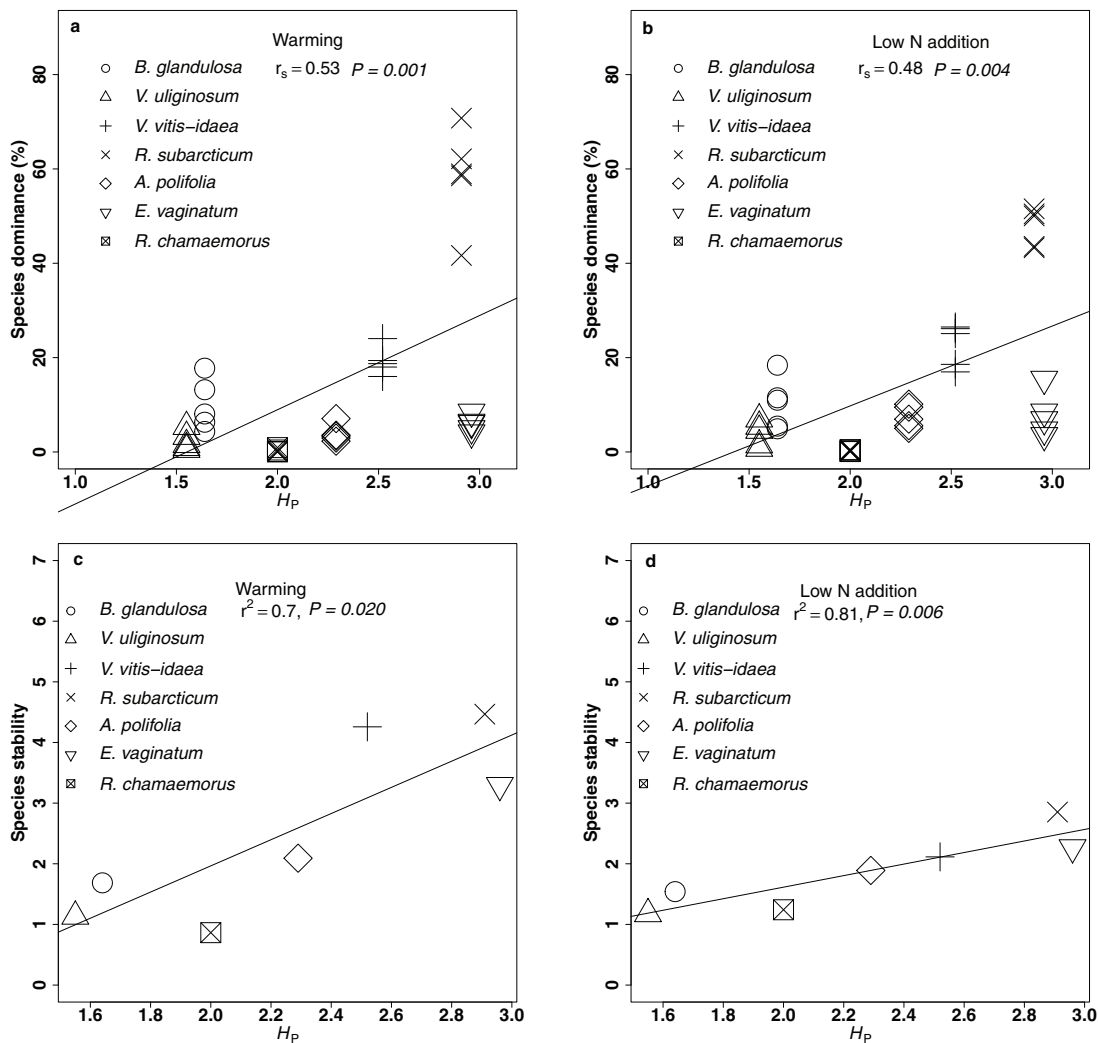
H index	Treatments	r_s	P
H_N -dominance	Control	-0.41	0.023
	Warming	-0.50	0.005
	Low N addition	-0.41	0.025
	High N addition	-0.43	0.016
	High P addition	-0.54	0.002
H_P -dominance	Control	0.51	0.002
	Warming	0.53	0.001
	Low N addition	0.48	0.004
	High N addition	0.39	0.021
	High P addition	0.39	0.021
$H_{N:P}$ -dominance	Control	0.56	0.001
	Warming	0.55	0.001
	Low N addition	0.53	0.001
	High N addition	0.13	0.456
	High P addition	0.35	0.042
H_P -stability	Warming	0.83	0.020
	Low N addition	0.90	0.006
$H_{N:P}$ -stability	Warming	0.82	0.025
	Low N addition	0.85	0.015
H_P -responsiveness	Low N addition	-0.83	0.021
$H_{N:P}$ -responsiveness	Low N addition	-0.79	0.036

Note: Spearman's rank correlation tests were used to calculate the correlation coefficients (r_s) and corresponding P values for the dominance data, as the assumption of normality was not met.

Stoichiometric H values for N differ among soil chemical forms of N

In our study, species H_N obtained with the different chemical forms of N varied substantially with each other, indicating that great caution is necessary when comparing H values between studies, as they may use different soil available nutrient chemical forms for the calculation of H . However, the consistency in species rankings among these indicators (i.e., species with high H_{TDN} also had high $H_{NH_4^+}$ and $H_{NO_3^-}$) suggests that H_N obtained from a particular N chemical form is a reasonable index of the relative strength of N homeostasis when comparing among species at a particular site, even though they differ in their relative uptake of different soil N chemical forms (McKane et al. 2002). Whether such consistency also occurs for P is unknown, as we assessed H_P using soil extractable PO_4^{3-} only. Like soil N, there is a wide range of chemical forms of soil P (especially as moderately labile polyphosphates and organically-bound orthophosphate diesters) that are available to tundra plants (Turner et al. 2004; Turner 2008; Weintraub 2011). Unfortunately, only inorganic phosphate data were available for this study's preliminary test of the applicability of the stoichiometric homeostasis concept. Clearly, further studies are needed to quantify the principal P forms other than simple phosphate, as well as their bioavailability, around individual target plants of each species so as to compare correlations among indices of H_P determined with different soil P forms and therefore to more comprehensively evaluate the potential influence of P-based stoichiometric homeostasis on plant community composition.

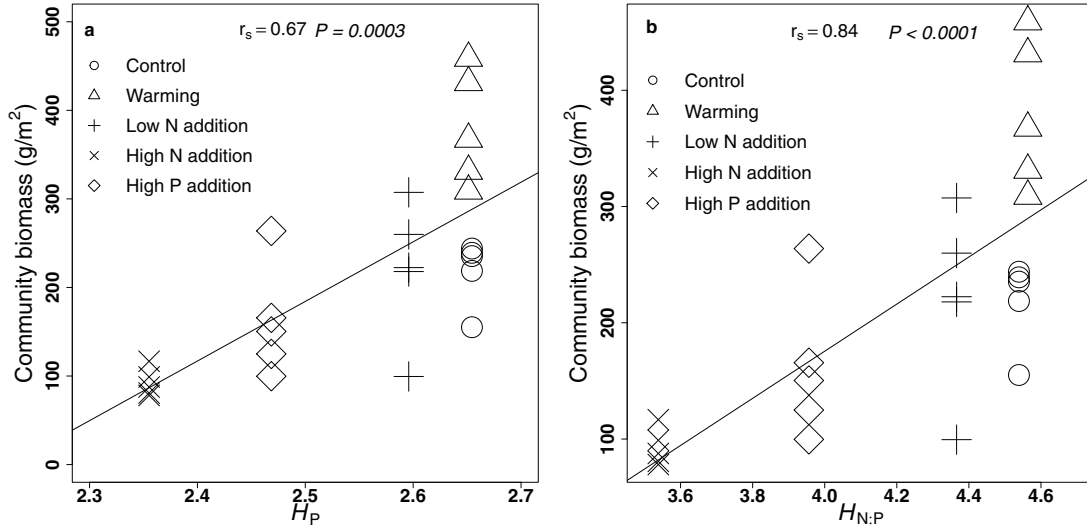
Fig. 2. Relationships between (a and b) species H_P and species dominance (% total aboveground biomass); and (c and d) species H_P and species spatial stability (the degree of constancy of aboveground biomass relative to its mean - see "Materials and methods" section) for the seven principal species in the warming plots and low N addition plots ($n = 5$). The sequence of species along the x-axes (i.e., increasing H_P) is as follows: *V. uliginosum*, *B. glandulosa*, *R. chamaemorus*, *A. polifolia*, *V. vitis-idaea*, *R. subarcticum*, and *E. vaginatum*. See Table 3 for the full data set across all experimental manipulations. Pearson's correlation (r^2) was used when the assumption of normality was met; otherwise, Spearman's rank correlation (r_s) was used.



Patterns in whole-shoot and tissue-specific H indices among species

Consistent with the view that ErM species are more efficient at accessing soil organic N and soil P than EcM species (Read et al. 2004; Turner 2008; Iversen et al. 2015), we found that the ErM species generally had higher H_N and H_P than the only EcM species (*B. glandulosa*). This result suggests that ericoid mycorrhizal fungi may be more efficient than ectomycorrhizal fungi in buffering their host plants' metabolism against variabilities in soil nutrient resources in low-fertility environments. Similar values of H among different tissues for a given species suggest considerable integration among plant tissues for nutrient homeostasis, which is consistent with a previous study showing that plant nutrient contents and N:P ratios were highly correlated across organs for both woody and herbaceous plants on a

Fig. 3. Relationships between (a) community H_P and community biomass and (b) community $H_{N:P}$ and community biomass across the five sets of experimental plots (sequence along x-axes: high N addition, high P addition, low N addition, warming, and control). Spearman's rank correlation (r_s) was used, as the assumption of normality was not met.



global scale (Kerkhoff et al. 2006; Elser et al. 2010). Our results indicate that although tundra species differ in woodiness, phenology, storage capacity, and many other life strategies (Chapin and Shaver 1988), foliar H is representative of the overall plant species homeostasis. Lastly, for the forb species *R. chamaemorus*, the unsuitability of the H model to its shoot N concentration perhaps reflects the fact that its growth in this ecosystem is primarily limited by soil P availability and does not respond to N addition (Zamin et al. 2014).

P may be a more important stoichiometric element than N in tundra ecosystems

In our study, H_P and $H_{N:P}$ rather than H_N were linked with species and community properties and responses to environmental changes, whereas previous studies in temperate grasslands found that H_N was the most powerful indicator (Yu et al. 2010, 2011, 2015). This difference may reflect the fact that growth of several of the most common species (i.e., *B. glandulosa* and *E. vaginatum*) at our tundra ecosystem study site were co-limited by N and P (Zamin and Grogan 2012; Zamin et al. 2014), while growth of most species in grassland ecosystems is primarily limited by N (Bai et al. 2010; Avolio et al. 2014). Additionally, different experimental durations between studies may also contribute to this inconsistency, as H_P also became important in predicting species dominance and stability at longer temporal scales (i.e. 27 years) in the grasslands (Yu et al. 2010).

The influence of species root to shoot ratio on stoichiometric homeostasis

Plant traits, such as root to shoot ratio and foliar nutrient concentrations, have been frequently associated with species performances in changing environments. Species with higher root mass may have a competitive advantage in responding to environmental variation (Tilman et al. 2006), and species with higher root to shoot ratios are reported as always having higher H values (Yu et al. 2010, 2015). Our study results challenge these conclusions. We previously collected both plant aboveground and belowground biomass in the warming and the corresponding control plots and estimated the root to shoot ratio for each species based on the living shoots and roots that could be identified to species (~23% and ~14% of the total root biomass was unidentifiable in control and warming plots, respectively) (Zamin et al. 2014). The most stoichiometrically homeostatic species (*R. subarcticum*) had

the lowest root to shoot ratio, while the least homeostatic species (*B. glandulosa*) had the highest root to shoot ratio among woody species in the control plots. Interestingly, after 8 years of experimental warming, the root to shoot ratio of *B. glandulosa* decreased considerably, while the root to shoot ratio of *R. subarcticum* was unchanged (Zamin et al. 2014). Meanwhile, the warming treatment increased the relative aboveground dominance of *R. subarcticum*, while *B. glandulosa* shoot biomass also increased by a similar proportion, but the absolute magnitude of the latter's increase was much less because of its relatively low biomass (Zamin et al. 2014). These results suggest that tundra species with high stoichiometric homeostasis do not necessarily have high root to shoot ratios, but have strong capability to maintain a stable root to shoot ratio under changing environments. Besides the high root to shoot ratio, low foliar N and P concentrations were also suggested as a characteristic of high-*H* plants (Yu et al. 2010, 2011). In our study, however, although foliar P concentrations were significantly lower in high-*H* species (e.g., *R. subarcticum*) than in low-*H* species (e.g., *B. glandulosa*), foliar N concentrations were similar. These results indicate that high-*H* species have more constrained concentrations of P compared to N and provide further support for the fundamental role of P in determining critical stoichiometric homeostasis effects in our tundra site at least.

Future directions

Our preliminary study indicates that stoichiometric homeostasis theory may provide a useful tool to describe the structure of mesic birch hummock tundra plant communities and to predict their responses to changing environmental conditions. Further research is now required to address the more general question: whether or to what extent *H* values obtained from a particular tundra ecosystem can be used to describe the species performances and community structure and functioning in other tundra ecosystems and (or) under different temporal scales. In grasslands, these kinds of larger scale temporal and spatial extrapolations were supported when relating *H* with dominance and temporal stability at the species level but not at the community level (Yu et al. 2010). In contrast with herbaceous grassland communities, tundra contains species that differ strongly in growth forms and life history strategies (Chapin et al. 1980; Chapin and Shaver 1988). Therefore, our study here should be interpreted as a starting point, and further extensive research may be required using longer-term nutrient addition experiments, natural environmental gradients of soil nutrient availability, multiple samplings throughout the growing season, and better indicators of soil nutrient supply to determine more robust *H* indices for relating stoichiometric homeostasis to species/community performances at extended temporal and spatial scales. Nevertheless, our preliminary study has successfully expanded stoichiometric homeostasis theory to a tundra ecosystem, and our results strongly suggest that stoichiometric homeostasis represents an innovative way to bridge fine-scale knowledge about plant traits with broader-scale community composition, structure, and functioning in a changing environment.

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