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A global trait-based approach to estimate leaf nitrogen functional allocation from observations

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Abstract. Nitrogen is one of the most important nutrients for plant growth and a major constituent of proteins that regulate photosynthetic and respiratory processes. However, a comprehensive global analysis of nitrogen allocation in leaves for major processes with respect to different plant functional types (PFTs) is currently lacking. This study integrated observations from global databases with photosynthesis and respiration models to determine plant-functional-type-specific allocation patterns of leaf nitrogen for photosynthesis (Rubisco, electron transport, light absorption) and respiration (growth and maintenance), and by difference from observed total leaf nitrogen, an unexplained "residual" nitrogen pool. Based on our analysis, crops partition the largest fraction of nitrogen to photosynthesis (57%) and respiration (5%) followed by herbaceous plants (44% and 4%). Tropical broadleaf evergreen trees partition the least to photosynthesis (25%) and respiration (2%) followed by needle-leaved evergreen trees (28% and 3%). In trees (especially needle-leaved evergreen and tropical broadleaf evergreen trees) a large fraction (70% and 73%, respectively) of nitrogen was not explained by photosynthetic or respiratory functions. Compared to crops and herbaceous plants, this large residual pool is hypothesized to emerge from larger investments in cell wall proteins, lipids, amino acids, nucleic acid, CO_2 fixation proteins (other than Rubisco), secondary compounds, and other proteins. Our estimates are different from previous studies due to differences in methodology and assumptions used in deriving nitrogen allocation estimates. Unlike previous studies, we integrate and infer nitrogen allocation estimates across multiple PFTs, and report substantial differences in nitrogen allocation across different PFTs. The resulting pattern of nitrogen allocation provides insights on mechanisms that operate at a cellular scale within leaves, and can be integrated with ecosystem models to derive emergent properties of ecosystem productivity at local, regional, and global scales.

Key words: *allocation; leaf; nitrogen; photosynthesis; plants; respiration; Rubisco; traits.*

Introduction

Nitrogen (N) is one of the important nutrients limiting plant growth (Vitousek and Howarth 1991, Reich et al. 2006, LeBauer and Treseder 2008), yet the representation of this limitation in land models used for climate change prediction is either missing or very uncertain (Zaehle and Dalmonech 2011, Zaehle et al. 2014). Nitrogen limitation increases with increasing latitude because of the less energetically favorable environment for nitrogen fixation (Vitousek and Howarth 1991, Houlton et al. 2008) as supported by analyses in chronosequences (Vitousek et al. 1993, Vitousek and Farrington 1997) and leaf-stoichiometry-based studies (Reich and Oleksyn 2004). Accurately characterizing plant responses to nitrogen limitation is critical for understanding terrestrial ecosystem responses over the next century, especially in relation to large-scale changes associated with increasing nitrogen deposition (Galloway and Cowling 2002, Matson et al. 2002), release of currently inaccessible soil nitrogen from permafrost degradation due to large-scale warming (Schuur et al. 2007, Natali et al. 2012), and rising atmospheric CO_2 concentrations that increases photosynthetic nitrogen use efficiency and provides a competitive advantage to nitrogen fixers (Ainsworth and Rogers 2007, Rogers et al. 2009).

Predicting plant responses to such large-scale changes requires a mechanistic understanding of nitrogen allocation for different plant processes (i.e., photosynthesis, respiration, growth), and also for cell structure and storage (Xu et al. 2012). Plants allocate nitrogen to produce enzymes and pigments that control these processes at a

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cellular scale (Evans 1989, Evans and Poorter 2001). These cellular-scale processes result in emergent local, regional, and global-scale controls on photosynthesis (Ehleringer and Field 1993, Chen et al. 1999, Reich 2012).

At leaf level, the capacity for CO_2 uptake is determined by nitrogen allocated to processes associated with light absorption, electron transport, and carboxylation (Farquhar et al. 1980, Evans 1989, Niinemets and Tenhunen 1997, Evans and Poorter 2001). Nitrogen is used in these processes as proteins to capture light energy in photosystems I/II, to drive the electron transport chain, and as Calvin cycle enzymes. In addition to photosynthesis, the mitochondrial enzymatic reactions that generate adenosine triphosphate (ATP) for maintenance and growth respiration is regulated by nitrogen availability (Wullschleger et al. 1992). Nitrogen is also required for maintaining cell structure and for storage compounds including reproduction and defense (Chapin et al. 1990).

The relative allocation of leaf nitrogen to these component processes and structures has been examined in detail for a few model species (Chapin et al. 1986, Evans 1989, Takashima et al. 2004, Xu et al. 2012) but little is known about how plants in natural ecosystems partition nitrogen resources, especially at regional and global scales. Some global land-surface models represent nitrogen limitation on photosynthesis by down-regulating potential photosynthesis rates if nitrogen is limiting (Oleson et al. 2013), assuming fixed maximum rate of carboxylation by the Rubisco enzyme (V_{cmax}) values for different plant functional types (PFTs; Moorcroft et al. 2001), predicting V_{cmax} from leaf nitrogen content based on prescribed V_{cmax}–leaf-nitrogen relationships (Zaehle and Friend 2010), or accounting for the carbon costs of nitrogen acquisition (Fisher et al. 2010). Although few modeling studies have incorporated varying nitrogen allocation within leaves (Zaehle and Friend 2010), the relationship of nitrogen allocation as a function of leaf nitrogen content is used to predict carbon fluxes and can be tuned to match carbon fluxes from flux tower data. In parallel, many studies have shown that plants allocate nitrogen near optimally among leaves across canopy depth (Field 1983, Hirose and Werger 1987, Ellsworth and Reich 1993) and within leaf enzymes (Medlyn 1996, Xu et al. 2012) to maximize productivity given environmental conditions. A mechanistic understanding of nitrogen controls on terrestrial vegetation processes can be improved and parameterized by characterizing leaf nitrogen allocation and how it varies between different PFTs, regionally and globally.

Nitrogen allocation in leaves likely varies because defense needs and environmental conditions vary, including carbon dioxide (CO_2), light, and temperature (Evans 1989). Regional differences in nitrogen allocation influence how plants respond to nitrogen deposition, with associated influences on photosynthesis and growth (Reich et al. 2001, Luo et al. 2004, Bobbink et al. 2010). The ability to synthesize regional leaf nitrogen allocation patterns and incorporate them into global land models has been hampered by sparse and isolated measurements of plant traits. Moreover, most global land models have constant photosynthesis parameters (e.g., V_{cmax}) that control photosynthesis, and nitrogen limitation occurs in these models by down-regulating (e.g., reducing) potential productivity rather than by dynamically simulating nitrogen allocation parameters based on nitrogen availability (Ghimire et al. 2016).

Few observational or modeling-based studies have reported the allocation of leaf nitrogen to a complete set of processes (including carboxylation, electron transport, light absorption, maintenance respiration, and growth respiration), and those that have focused on relatively few samples. Leaf nitrogen varies with environmental conditions, leaf traits, and geographic location (Reich et al. 1992, 1997, Reich and Oleksyn 2004, Wright et al. 2004, 2005), and is correlated with photosynthetic parameters (Wullschleger 1993, Xu and Baldocchi 2003, Coste et al. 2005, Grassi et al. 2005). Studies have determined the fractional allocation of leaf nitrogen among proteins and the associated influence on individual process rates (e.g., Evans 1989, Niinemets and Tenhunen 1997, Onoda et al. 2004, Takashima et al. 2004). However these studies relied on a few measurements (i.e., data at only three sites) for evaluating the behavior of their optimal nitrogen allocation model (e.g., Xu et al. 2012), considered limited PFTs (mostly short-lived non-woody plants or woody juveniles) that lacked explicit representation of interspecies difference in nitrogen allocation (e.g., Chapin et al. 1986, Evans 1989, Makino and Osmond 1991, Onoda et al. 2004, Takashima et al. 2004, Guan and Wen 2011), or did not consider nitrogen allocation for a complete range of processes including carboxylation, light harvesting, bioenergetics, maintenance respiration, and growth respiration (e.g., Coste et al. 2005, Delagrange 2011). Higher nitrogen investments in photosynthesis and respiration are expected for crops followed by herbaceous plants and then longer-lived woody PFTs (Chapin et al. 1986, Evans 1989, Takashima et al. 2004) but the relative magnitudes of the nitrogen partitioning to different functions (especially for tropical and temperate trees) at a global scale is currently lacking. Plants invest the largest proportion of nitrogen for photosynthesis to Rubisco, followed by light absorption and electron transport with allocation sensitive to environmental conditions (Evans and Seemann 1989, Takashima et al. 2004, Guan and Wen 2011). Compared to photosynthesis, the nitrogen partitioning to respiration is lower (4–7%) for crops (Makino and Osmond 1991), but little is known about the respiratory nitrogen allocation for other PFTs.

In this study, we provide a comprehensive analyses of leaf nitrogen allocation for a range of PFTs at a global scale by synthesizing observations in the TRY database (Kattge et al. 2011) with observations from a high-latitude Arctic coastal tundra ecosystem (Rogers 2014; A. Rogers, *unpublished data*). We undertake a process-level

representation of leaf nitrogen allocation for a range of leaf processes including carboxylation (e.g., Rubisco), light capture, electron transport, maintenance respiration, and growth respiration. We hypothesize that the fractional partitioning of leaf nitrogen to these processes varies with leaf nitrogen content due to changes in plant strategies as leaf nitrogen availability increases. We also estimate how leaf nitrogen allocation varies across a range of PFTs that have differing photosynthetic nitrogen use efficiency and growth and survival strategies. An important goal of this work is to develop a leaf nitrogen allocation framework that can be integrated into Earth System Models (ESMs) to contribute to a dynamic representation of leaf characteristics. The varying fractional nitrogen allocation within leaves for different processes can be used in ESM's to derive photosynthetic parameters (such as V_{cmax} and maximum electron transport rate J_{max}), leaf pigment content (e.g., chlorophyll), and respiration related parameters.

Materials and Methods

Our goal in this study is to quantify nitrogen allocation in leaves, by combining either observationally inferred (for electron transport rate and Rubisco capacity) or modeled (light capture and respiration) leaf function with the observed stoichiometric nitrogen ratios required to support that functioning. We also quantify nitrogen allocated to a residual pool, which we assume supports other processes not explicitly represented in this study (e.g., defense). We then assess overall patterns of nitrogen allocation, and compare how nitrogen is allocated to the resolved and unresolved processes.

Plant trait data to quantify nitrogen allocation in leaves were obtained from the TRY and Next-Generation Ecosystem Experiments (NGEE Arctic) databases. The TRY database (Kattge et al. 2011) is a compilation of several original trait databases: this study uses data via TRY from 24 original data sets (Table 1). We have attempted to access as much data as possible from the TRY database based on approval of data providers but note that our sample of data does not represent the entire data available in the TRY database because multiple variables used in this study for the same observational unit are not always available. Our data set has relatively more samples in mid-latitude and tropical regions compared to high latitude Arctic ecosystems. We therefore additionally acquired data collected by the NGEE-Artic project in Barrow, Alaska that includes data for herbaceous PFTs in the Arctic.

We integrate observations of leaf nitrogen with photosynthetic and respiratory parameters and rates using a simple model of nitrogen function in leaves to disaggregate the leaf nitrogen into functional pools in different

Table 1. Sources for plant traits used from the TRY database for different plant functional types (PFTs).

Source	PFT	V_{cmax}	J_{max}	Leaf N
Harley et al. (1992)	CRP	×	×	×
U. Grueters, *unpublished data*	CRP	×	×	×
Ellsworth et al. (2004)	HRB; SRB; BDT; NET	×	×	×
Meir et al. (2007)	HRB; BDT; BET	×	×	×
Tissue et al. (1995)	HRB	×	×	×
J. Kattge, *unpublished data*	HRB; SRB; BDT; NET	×	×	×
Wohlfahrt et al. (1999)	HRB; SRB	×	×	×
Domingues et al. (2007)	SRB; BET	×		×
Ecocraft Database 1999 (Medlyn and Jarvis 1997)	SRB; BDT; BET; NET	×	×	×
Whitehead et al. (2004)	SRB	×	×	×
Wullschleger (1993)	SRB	×	×	×
Dungan et al. (2003)	BDT; BET	×	×	×
Le Roux et al. (1999)	BDT	×	×	×
Medlyn et al. (1999)	BDT; NET	×		×
Meir et al. (2002)	BDT; BET; NET	×		×
Kattge (2002)	BDT; BET	×	×	×
Ripullone et al. (2003)	BDT	×	×	×
Walcroft et al. (2002)	BDT	×	×	×
Coste et al. (2005)	BET	×	×	×
Domingues et al. (2005)	BET	×		×
Kumagai et al. (2006)	BET	×	×	×
Han et al. (2004)	NET	×	×	×
Tissue et al. (1999)	NET	×	×	×
Walcroft et al. (1997)	NET	×	×	×

Notes: CRP, crops; HRB, herbaceous plants; SRB, shrubs; BDT, broadleaf deciduous trees; BET, broadleaf evergreen trees; NET, needle-leaved evergreen trees. V_{cmax} is the maximum rate of carboxylation by the Rubisco enzyme, and J_{max} is the maximum electron transport rate

PFTs. The data used in this analysis are attributed to different PFTs using the lookup table available from the TRY website.[9] In our analysis, nitrogen is allocated to different leaf-level processes: photosynthesis, respiration, and residual (i.e., the remaining leaf nitrogen not allocated to photosynthesis and respiration). Leaf nitrogen allocated to photosynthesis is further divided into light absorption, electron transport, and Rubisco enzyme. Lastly, leaf nitrogen allocated to respiration is further divided into growth and maintenance respiration. This approach to estimate leaf nitrogen allocated to growth respiration does not explicitly account for leaf age, but the effects of leaf age are implicit in this approach as mature leaves have lower nitrogen, which would reduce gross assimilation and nitrogen allocation for growth, compared to younger leaves in our study. Leaf nitrogen allocated to respiration refers to nitrogen in mitochondrial enzymes associated with mitochondrial respiration for maintenance and growth of plant tissues. We inferred the nitrogen allocation to these processes from the traits (i.e., V_{cmax}, J_{max}, and leaf nitrogen) and used a photosynthesis and respiration model when observations corresponding to certain processes were lacking.

Our approach to partitioning leaf photosynthesis nitrogen to light absorption, electron transport, and Rubisco is based on Niinemets and Tenhunen (1997), and has been used by several studies (e.g., Grassi and Bagnaresi 2001, Le Roux et al. 2001, Walcroft et al. 2002, Han et al. 2003, Ripullone et al. 2003, Coste et al. 2005). Leaf nitrogen partitioning to carboxylation (mainly Rubisco; P_R) is determined based on the approach by Niinemets and Tenhunen (1997)

$$P_R = \frac{V_{cmax}}{6.22 V_{cr} N_a} \tag{1}$$

where V_{cmax} (μmol CO_2·m leaf^{-2}·s^{-1}) is the maximum rate of carboxylation by the Rubisco enzyme, V_{cr} (μmol CO_2·g Rubisco^{-1}·s^{-1}) is the specific activity of Rubisco, N_a is the leaf nitrogen content (g N/[m leaf]^2), and 6.22 g Rubisco/g N in Rubisco converts nitrogen content to protein content (Rogers 2014). At leaf temperature of 25°C, V_{cr} reported in the literature ranges from 20.78 μmol CO_2·g Rubisco^{-1}·s^{-1} (Niinemets and Tenhunen 1997) to 47.3 μmol CO_2·g Rubisco^{-1}·s^{-1} (Rogers 2014), and we estimated the uncertainty in nitrogen allocation for different processes by applying both these V_{cr} values. V_{cmax} is obtained from the maximum rate of carboxylation by the Rubisco enzyme data reported in the TRY database and further standardized to 25°C based on Kattge and Knorr (2007), and N_a is obtained from the leaf nitrogen content (g N/[m leaf]^2) reported in the TRY database.

Leaf nitrogen partitioning to bioenergetics (i.e., electron transport) (P_B) is determined based on the method proposed by Niinemets and Tenhunen (1997)

$$P_B = \frac{J_{max}}{8.06 J_{mc} N_a} \tag{2}$$

where J_{max} (μmol electron·m leaf^{-2}·s^{-1}) is the maximum electron transport rate, J_{mc} (μmol electron·μmole cytochrome f^{-1}·s^{-1}) is the potential rate of photosynthetic electron transport per unit cytochrome f, and 8.06 (μmol cytochrome f/g N in bioenergetics) converts nitrogen content to protein content (Niinemets and Tenhunen 1997). At leaf temperature of 25°C, J_{mc} equals 156 μmol electron·μmol cytochrome f^{-1}·s^{-1} (Niinemets and Tenhunen 1997) and J_{max} is obtained from the maximum electron transport rate standardized to 25°C based on Kattge and Knorr (2007).

Leaf nitrogen partitioning to light absorption by chlorophyll (P_L) is determined based on the approach of Niinemets and Tenhunen (1997) and is estimated as

$$P_L = \frac{C_C}{C_B N_a} \tag{3}$$

where C_C is the chlorophyll content (mmol chlorophyll/[m leaf]^2) and C_B (1.78 mmol chlorophyll g^{-1} N in chlorophyll) is the amount of nitrogen in chlorophyll molecule (i.e., chlorophyll binding coefficient; Niinemets and Tenhunen 1997). At present, C_c values are scarce, especially corresponding to data points where V_{cmax} and J_{max} data exist. As a result, we estimate C_c by assuming that plants optimally allocate nitrogen for light absorption and electron transport processes, such that these processes proceed at the same rate (Xu et al. 2012). To assess the uncertainty of this assumption, we consider an additional scenario where these process rates proceed at 80% efficiency, such that the electron transport rate equals 80% of the light absorption rate. The details of the equations used in estimating C_c are provided in Appendix S1. Based on the optimal nitrogen allocation assumption, C_c is calculated from the electron transport rate and photosynthetically active radiation (PAR) from a 1° spatial resolution three-hourly surface downward shortwave radiation meteorological forcing data set (Sheffield et al. 2006) as described in Appendix S2. We extracted temperature and PAR at each location based on the spatial location of the observations. The uncertainties in nitrogen allocation for efficiency of light absorption and electron transport processes, leaf light exposure, and plant traits are reported as means and standard deviations.

In addition to photosynthesis, leaf respiration is a critical component of plant growth and survival. Nitrogen is used in mitochondrial cellular respiratory enzymes to produce energy (i.e., ATP) by oxidizing the products of photosynthesis (Makino and Osmond 1991). The two major components of respiration are maintenance and growth respiration. Maintenance respiration is associated with a range of processes including protein turnover and synthesis, maintenance of ionic and metabolite gradients, and membrane repair (Ryan 1991*a*, Amthor

[9] http://www.try-db.org/TryWeb/Data.php

2000). Nitrogen is allocated to mitochondrial enzymes that regulate these processes (Makino and Osmond 1991). Leaf nitrogen partitioning for maintenance respiration (P_M) is estimated as

$$P_M = \frac{R_M}{R_s N_a} \quad (4)$$

where R_M (μmol CO_2·m leaf^{-2}·s^{-1}) is the rate of maintenance respiration and R_s (μmol CO_2·g mitochondrial N^{-1}·s^{-1}) is the enzyme activity per unit of mitochondrial protein. At leaf temperature of 25°C, R_s equals 33.69 μmol CO_2·g mitochondrial N^{-1}·s^{-1} (Makino and Osmond 1991, Xu et al. 2012) and R_M is estimated based on Ryan (1991*b*) as

$$R_M = R_b N_a \quad (5)$$

where R_b (0.30 μmol CO_2·g N^{-1}·s^{-1}) is the base rate of maintenance respiration per unit nitrogen at 25°C (Ryan 1991*b*, Oleson et al. 2013).

The second major component of leaf respiration, growth respiration, involves enzyme-mediated growth of new tissues from the photosynthetically fixed carbon, including building tissues involved in photosynthesis, respiration, defense, and cell structure but excluding nitrogen directly invested in regulating these processes. Growth respiration is associated with the metabolic energy used in the construction of organic compounds from substrates (Ryan 1991*a*). Nitrogen is allocated to mitochondrial enzymes that regulate these processes (Makino and Osmond 1991). Leaf nitrogen partitioning for leaf growth respiration (P_G) is estimated as

$$P_G = \frac{R_G}{R_s N_a} \quad (6)$$

where R_G (μmol CO_2·m leaf^{-2}·s^{-1}) is the rate of growth respiration and R_s (μmole CO_2·g mitochondrial N^{-1}·s^{-1}) is the enzyme activity per unit of mitochondrial protein. At a leaf temperature of 25°C, R_s equals 33.69 μmol CO_2·g mitochondrial N^{-1}·s^{-1} (Makino and Osmond 1991, Xu et al. 2012). This approach to estimate leaf nitrogen allocated to growth respiration (R_G) does not explicitly account for leaf age (i.e., maturity; because the TRY database does not explicitly specify leaf age), but the effects of leaf age is implicit in our approach as mature leaves have lower nitrogen, which would reduce gross assimilation and nitrogen allocation for growth, compared to younger leaves in our study. We leave an explicit characterization of the impacts of leaf age on respiration to future work. R_G is determined as a fixed fraction of gross assimilation (Ryan 1991*a*, *b*) and is standardized to 25°C based on the temperature response function for respiration as implemented in the Community Land Model version 4.5 (CLM4.5; Oleson et al. 2013)

$$R_G = f_G f_L A_g \quad (7)$$

where A_g (μmol CO_2·m leaf^{-2}·s^{-1}) is gross assimilation, f_G (0.25) is the fraction of A_g partitioned to growth respiration (Williams et al. 1987, Ryan 1991*a*, *b*), and f_L (0.33) is the fraction of A_g allocated to the leaf (Potter et al. 1993, Malhi et al. 2011). The constant value for fraction of A_g partitioned to growth respiration is consistent with several studies, which have shown that respiration is usually a fixed fraction of productivity (Waring et al. 1998, Gifford 2003, Vicca et al. 2012). A_g is estimated by coupling the Farquhar photosynthesis model with the Ball Berry stomatal conductance model as described in Appendix S3. Meteorological forcing (i.e., temperature, relative humidity and PAR) for driving the Farquhar photosynthesis model is described in Appendix S2.

We assume the remaining leaf nitrogen unallocated to photosynthesis and respiration is allocated as residual nitrogen. Residual nitrogen includes nitrogen used in genetic material (i.e., DNA and RNA), cell structure, defense, and storage for reuse at later time. The residual pool also includes inactive Rubisco, and enzymes other than Rubisco that are involved in carboxylation. Using the relationships and observations described above, we present the fractional nitrogen allocation for broadleaf deciduous trees, broadleaf evergreen trees, needle-leaved evergreen trees, crops, shrubs, and herbaceous PFTs.

We provide assessment of the uncertainties associated with trait and climate uncertainties in our nitrogen allocation scheme. As described above, we estimate net carbon assimilation from the Farquhar model (Farquhar et al. 1980) driven separately for two light conditions (low and mean PAR), mean temperature conditions, and mean humidity condition. We considered nitrogen allocation for mean temperature, light, and humidity conditions computed as leaf are index (LAI) weighted daily daytime mean of hourly temperatures, PAR, and humidity from 1990 to 2005, respectively. The LAI weighting of temperature, PAR, and humidity is performed over each day by weighting the daily daytime mean of hourly temperature, PAR, and humidity, respectively, with the corresponding monthly LAI for the given day. Further details of the calculations of the environmental conditions are provided in Appendix S2. In addition to the uncertainty in nitrogen allocation attributed to leaf light exposure, we also report variation in allocation associated with trait variability by bootstrap-based sampling (i.e., sampling 75% of the data repeatedly with replacement, and reporting the mean and standard deviation of the repeated samples) of plant traits (e.g., leaf nitrogen and allocation to different processes) rather than using the mean value of plant traits, which would ignore the variability within PFTs. We calculated uncertainties for all the different scenario combinations and then calculated the mean and standard deviation based on these scenario combinations. Furthermore, ANOVA was performed to test for significant differences in nitrogen

allocation among different PFTs and leaf level processes for samples reported in the TRY database for combinations of PFTs and leaf level processes inferred using mean temperature, light, and relative humidity conditions. The regression slopes and intercepts of the fraction of leaf nitrogen allocation to Rubisco vs. leaf nitrogen are derived using the ordinary least squares (OLS) method. However the intercept and slopes of these relationships could be artifacts of the positive intercepts of the V_{cmax} and leaf nitrogen relationship. We therefore also recomputed the slopes and intercepts of these plant functional type dependent relationships by simultaneously fitting to both the V_{cmax} and leaf nitrogen relation (forcing an intercept of zero) as well as the fraction of leaf nitrogen allocation to Rubisco and leaf nitrogen relation using a Bayesian Markov chain Monte Carlo (MCMC) method.

Results

Leaf nitrogen allocation to the different leaf processes varied by PFT (Fig. 1), with the error bars representing the standard deviation about the mean. For crops, the total nitrogen allocation to the functionally explained pools (i.e., photosynthesis plus respiration) was 1.61 times larger than nitrogen allocation to the residual pools (which are processes that do not have a clearly defined function in the context of our analysis), primarily because of the large investment in photosynthesis, and 0.93 times that of herbaceous plants. The total nitrogen allocation to functionally explained pools relative to residual pools (i.e., ratio of functionally explained pools to residual pools) are about similar (i.e., 0.80) for broadleaf deciduous trees and non-tropical broadleaf evergreen trees. In the three other PFTs (shrubs, needleleaf evergreen trees, tropical broadleaf evergreen trees), the ratio of total allocation to functionally explained pools vs. the residual pools ranged from 0.70 for shrubs to 0.36 for tropical broadleaf evergreen trees, respectively. Across all PFTs, the nitrogen allocation to respiration was smaller than the nitrogen allocation to photosynthesis.

For photosynthesis, the largest leaf nitrogen allocation was for Rubisco followed by light absorption and electron transport. The uncertainty in the nitrogen allocation to light absorption is mostly due to trait variations and partly due to uncertainty in the light conditions that leaves were exposed to at the site and that we used in our photosynthesis model. In low light conditions, leaf nitrogen allocation to light absorption is increased in order to capture more light. This increase leads to a corresponding decrease in the nitrogen residual pool, which represents the excess leaf nitrogen after use by photosynthesis and respiration. Conversely, in higher light conditions, leaf nitrogen allocation to light absorption decreased compared to allocation to Rubisco. The total amount of leaf nitrogen allocation to residual and photosynthesis was highest in needle-leaved evergreen trees (predominately found in higher latitudes; Fig. 1). This pattern of increasing leaf nitrogen content with increasing distance from the equator has been reported by Reich and Oleksyn (2004).

The patterns of fractional leaf nitrogen allocation to the different processes varied among PFTs (Fig. 2) with the ANOVA F statistic (107; df 4079) significantly different ($P < 0.001$). Post hoc tests across combinations of PFTs and leaf functions showed that the nitrogen fraction allocated to the residual pool was the largest

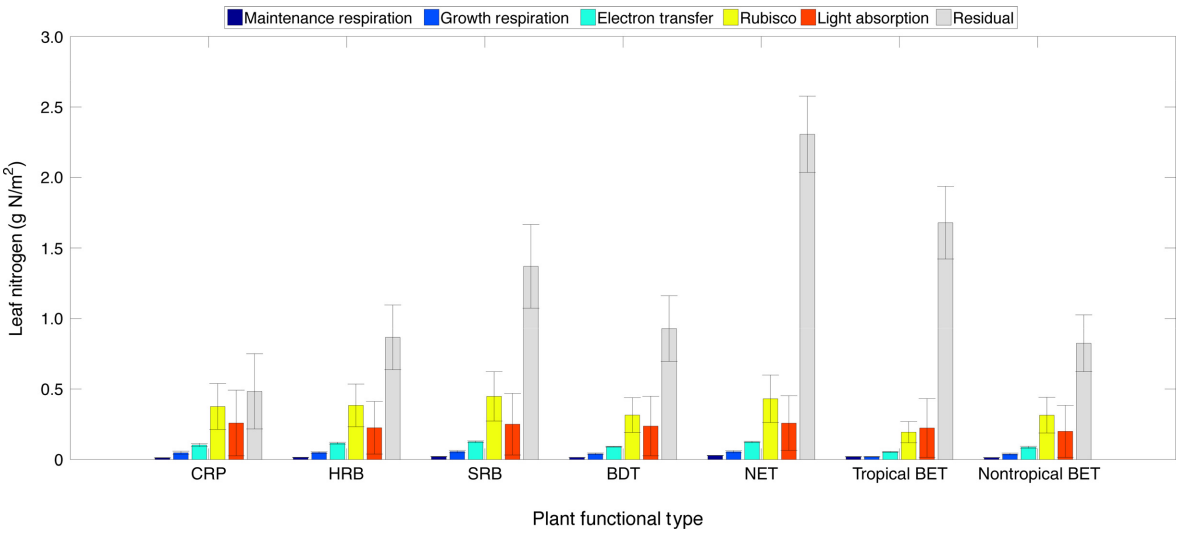

Fig. 1. Inferred leaf nitrogen allocated to maintenance respiration, growth respiration, electron transport, Rubisco, light absorption, and residual in the plant functional types analyzed from the TRY database. Plant functional types are described in Table 1. The error bars represent standard deviation about the mean. [Color figure can be viewed at wileyonlinelibrary.com]

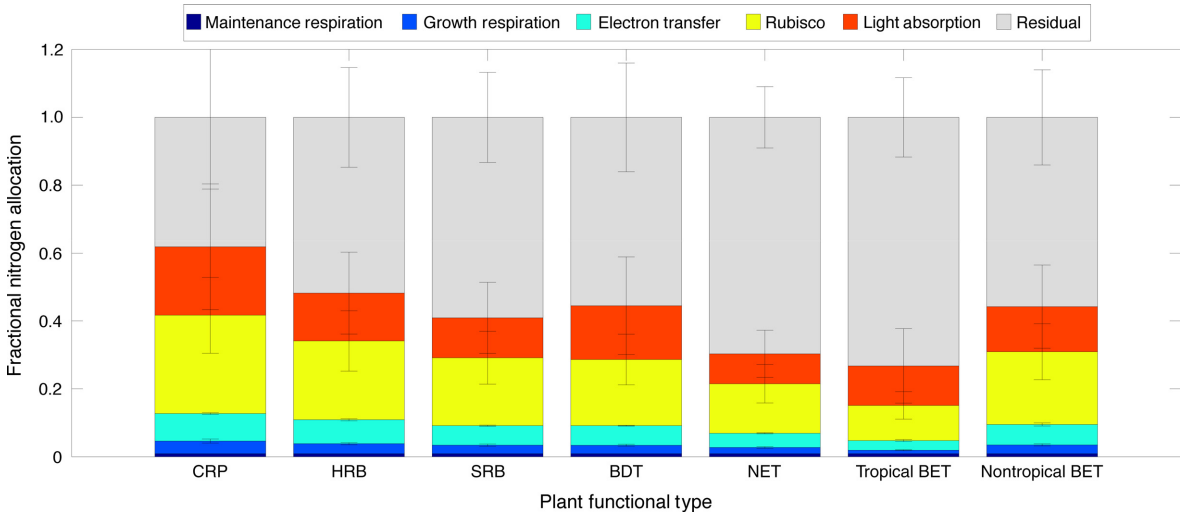

FIG. 2. Fractional leaf nitrogen allocation for maintenance respiration, growth respiration, electron transport, Rubisco, light absorption, and residual in the plant functional types analyzed from the TRY database. Plant functional types are described in Table 1. The error bars represent standard deviation about the mean. [Color figure can be viewed at wileyonlinelibrary.com]

difference among different PFTs followed by nitrogen fraction allocated to Rubisco. In addition, the absolute leaf nitrogen allocation to the different processes also varied among PFTs with the ANOVA F statistic (76; df 4079) significantly different ($P < 0.001$). As expected, crop PFTs have the greatest fractional leaf nitrogen allocation to photosynthesis (i.e., 8% of leaf nitrogen for electron transport, 29% of leaf nitrogen for Rubisco, 20% of leaf nitrogen for light absorption), consistent with their relatively quick growth rates and genetic modifications to maximize production compared to other nitrogen demands (e.g., defense). The fractional leaf nitrogen allocation to Rubisco, the most important parameter regulating photosynthesis, is lowest in tropical broadleaf evergreen trees (10% of leaf nitrogen).

Leaf nitrogen content was highly correlated with photosynthetic sub-processes and their parameterized representations. V_{cmax} and J_{max} both increased with increases in leaf nitrogen when all PFTs were grouped together (Fig. 3a,b). The equations in Fig. 3 are determined by forcing the regression through an intercept of zero because if nitrogen is absent, the V_{cmax} and J_{max} values should be zero. This increasing relationship of V_{cmax} and J_{max} with increases in leaf nitrogen has been shown by

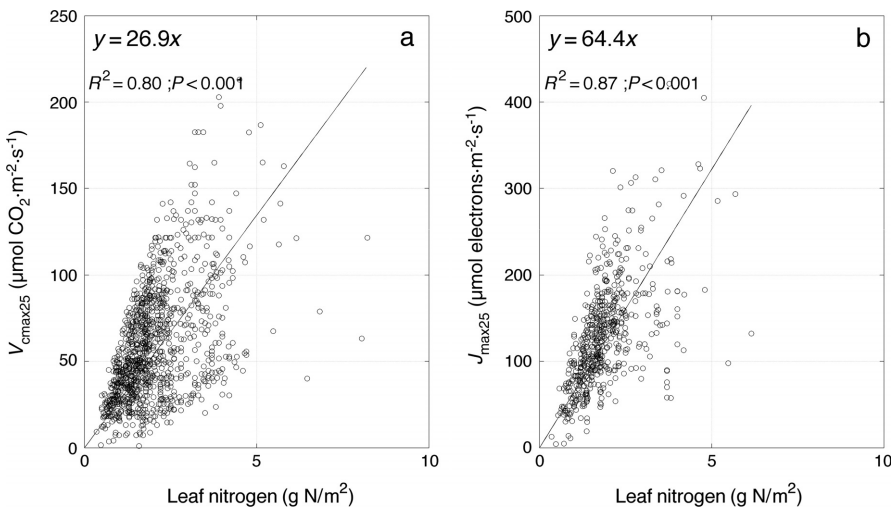

FIG. 3. Relationships for all plant functional types between leaf nitrogen content (N) and (a) the maximum rate of carboxylation by the Rubisco enzyme at the reference temperature of 25°C (V_{cmax25}) and (b) the maximum electron transport rate at the reference temperature of 25°C (J_{max25}). The linear regression equation was fitted with an intercept of 0.

several studies in individual forest and grassland systems (Wilson et al. 2000, Ripullone et al. 2003, Han et al. 2004, Takashima et al. 2004, Kattge et al. 2009).

However, the fraction of leaf nitrogen allocation to Rubisco with increasing leaf nitrogen increased for crops, remained almost unchanged for shrubs (comprised of 79% evergreen and 21% deciduous species) and broadleaf deciduous trees, and decreased for herbaceous plants, broadleaf evergreen trees, and needle-leaved evergreen trees (Fig. 4). Nitrogen allocation differences among PFTs and greater variation in the fraction of leaf nitrogen allocation to Rubisco is observed within plant functions types under low leaf nitrogen rather than under high leaf nitrogen conditions. This pattern may reflect variations due to different plant strategies, nitrogen use efficiencies, and adaptation of species to site-specific environmental conditions (e.g., light, moisture, temperature, and humidity). At high leaf nitrogen there is lower variability, suggesting that most of these plants invest excess nitrogen for functions other than photosynthesis. However we cannot make a conclusive statement of lower variability at high leaf nitrogen because of lower sample size at higher leaf nitrogen compared to lower leaf nitrogen. Future research should investigate differences in nitrogen allocation among PFTs. The recomputed slopes and intercepts determined by MCMC (to minimize the influence of the artifacts of the positive intercepts of the V_{cmax} and leaf nitrogen relationship as described in the materials and methods section) have the same value as the slopes (i.e., 0.07 for crops, −0.07 for herbaceous plants, −0.06 for broadleaf evergreen trees, and −0.04 for needle-leaved evergreen trees) and intercepts (i.e., 0.27 for crops, 0.40 for herbaceous plants, 0.29 for broadleaf evergreen trees, and 0.32 for needle-leaved evergreen trees) determined using OLS (see Fig. 4), implying that the slope and intercepts in Fig. 4 are not artifacts of the positive intercept of the V_{cmax} and leaf nitrogen relationship. The correlations between fractional leaf nitrogen allocation pools, photosynthesis parameters, and leaf nitrogen for all PFTs combined are shown in Fig. 5. The cluster of positively correlated variables is displayed in blue (bottom half of Fig. 5) and the cluster of negatively correlated variables is displayed in brown-red (top of Fig. 5).

Discussion

Fractional allocation of leaf nitrogen to various processes (e.g., photosynthesis, respiration, and residual) varies by PFT. In this study, we synthesized leaf nitrogen trait data from the TRY database (Kattge et al. 2011) and a high-latitude Arctic coastal tundra ecosystem study (Rogers 2014; A. Rogers, *unpublished data*), and integrated these data with a photosynthesis and respiration sub-model. We used the data and model to estimate leaf nitrogen allocation for these various processes at a

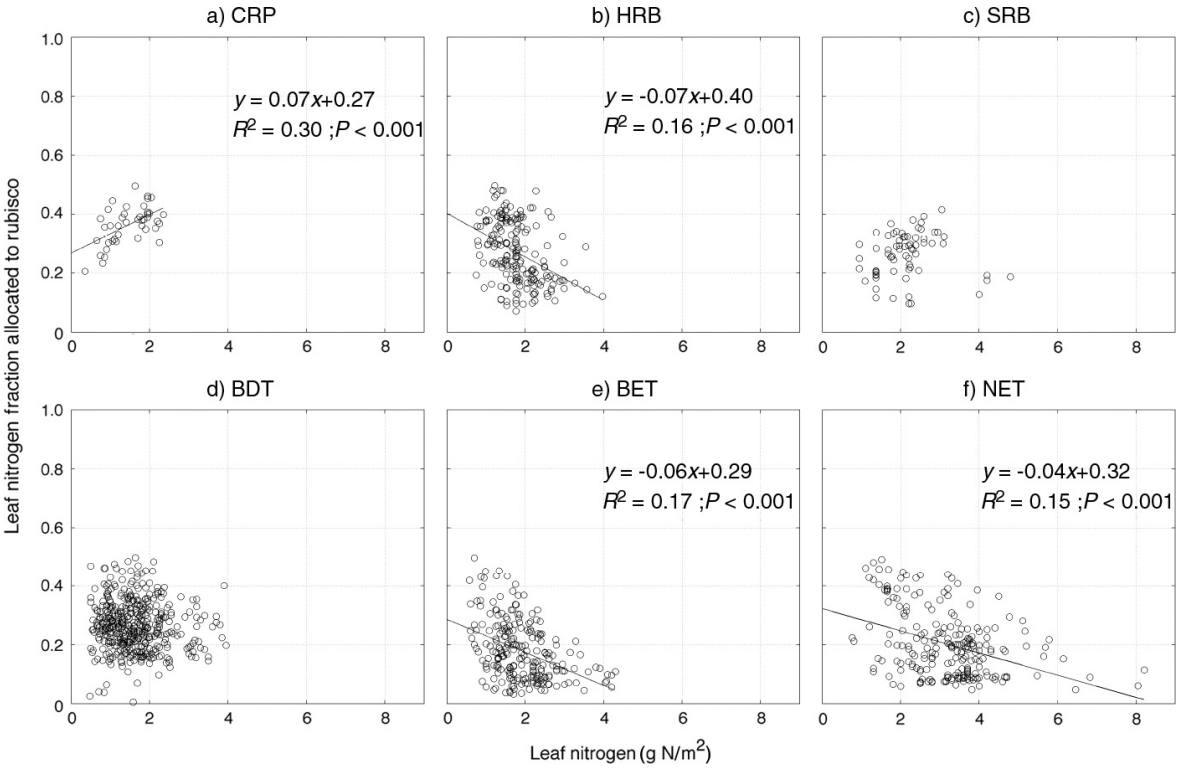

Fig. 4. Relationships between leaf nitrogen and the leaf nitrogen fraction allocated to Rubisco for (a) crops, (b) herbaceous plants, (c) shrubs, (d) broadleaf deciduous trees, (e) broadleaf evergreen trees, and (f) needleaf evergreen trees.

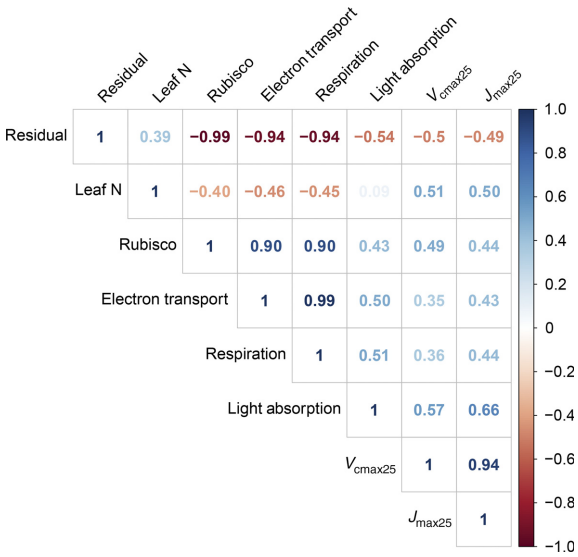

FIG. 5. The correlations between fractional leaf nitrogen allocation pools, photosynthesis parameters, and leaf nitrogen for all plant functional types combined. Electron transport is fractional leaf nitrogen allocation for electron transport, Rubisco is fractional leaf nitrogen allocation for Rubisco, light absorption is fractional leaf nitrogen allocation for light absorption, respiration is fractional leaf nitrogen allocation for growth and maintenance respiration, residual is remaining fractional leaf nitrogen not allocated to photosynthetic and respiratory processes, leaf N is leaf nitrogen, V_{cmax25} is the maximum rate of carboxylation by the Rubisco enzyme at the reference temperature of 25°C, and J_{max25} is the maximum electron transport rate at the reference temperature of 25°C. [Color figure can be viewed at wileyonlinelibrary.com]

global scale. The resulting pattern of nitrogen allocation provides insights on mechanisms that operate at a cellular scale within leaves, and can be integrated with ecosystem models to derive emergent properties of ecosystem productivity at local, regional, and global scales. We conclude that existing ecosystem models can be improved by representing each of these processes as functional nitrogen pools within the leaf. The allocation patterns presented in this study can be used to calibrate or evaluate these improved models having functional nitrogen pools. The varying fractional nitrogen allocation within leaves for different processes can be used in ESMs to derive photosynthetic parameters (such as V_{cmax} and J_{max}), leaf pigment content (e.g., chlorophyll), and respiration related parameters. For example, models with a prognostic leaf nitrogen pool (e.g., a version of CLM4.5 we developed [Ghimire et al. 2016]) can predict variations in photosynthetic parameters with changes in leaf nitrogen. The allocation patterns reported in this study can be used to evaluate models (e.g., OC-N; Zaehle and Friend 2010) that predict the variation in allocation within leaves.

In our partitioning scheme, the nitrogen partitioning to photosynthesis is lowest in tropical broadleaf evergreen forests (25%) compared to other PFTs. These tropical trees have low photosynthetic nitrogen use efficiencies (Kattge et al. 2009) and are located on phosphorus-depleted oxisol soils. Therefore, we hypothesize that phosphorous limitation reduces the benefit of larger nitrogen investments to photosynthesis. In contrast, crops have the greatest fractional leaf nitrogen allocation to photosynthesis (57%; i.e., sum of nitrogen for Rubisco, electron transfer and light absorption) in comparison to other PFTs, resulting in higher growth and productivity. Our estimate for crops is comparable to the range of around 50% (shade leaf) to 60% (sun leaf) reported by Evans and Seemann (1989). Takashima et al. (2004) examined evergreen and deciduous species of the genus *Quercus* and found that deciduous species invest higher proportion of leaf nitrogen to photosynthesis (40%) compared to evergreen species (30%), and attributed this difference to greater allocation to cell walls in evergreen species. Although it is difficult to compare directly to Takashima et al. (2004) because our analysis is based on observations for many species, we found similar partitioning to photosynthesis (41% in our study compared to 40%) for deciduous PFTs (i.e., broadleaf deciduous trees), comparable partitioning (28% and 25% in our study compared to 30%) to photosynthesis for evergreen PFTs (i.e., needle evergreen trees and tropical broadleaf evergreen trees), and greater allocation to photosynthesis (41% compared to 30%) for non-tropical broadleaf evergreen trees compared to Takashima et al. (2004). A possible interpretation of these results is that the longer leaf longevity of evergreen trees requires a larger fraction of nitrogen to be invested in structural and defensive compounds in order to support the longer-lived leaves.

Across most PFTs in our study, the largest proportion of nitrogen for photosynthesis is allocated to Rubisco, followed by light absorption and electron transport; these results are consistent with previous studies (Evans and Seemann 1989, Takashima et al. 2004, Guan and Wen 2011). Also, our estimates of the impacts of light levels on nitrogen allocation to light absorption is consistent with several previous studies (Evans 1989, Niinemets et al. 1998), with higher light availability causing reduced nitrogen investment to light absorption and vice versa. The respiratory mitochondrial protein partitioning of 5% for crops in our study is consistent with the partitioning of 4% to 7% estimated for crops (Makino and Osmond 1991).

The total range of variation for the residual pools across all PFTs considered in this study is around 38% for crops to 73% for tropical broadleaf evergreen trees. Assuming that respiratory mitochondrial enzymes constitute 5% of total leaf nitrogen, a study by Evans and Seemann (1989) estimated a range of 35% (sun leaf) to 55% (shade leaf) nitrogen partitioning to residual pools for crops, which is comparable to the estimate for crops in our study. Takashima et al. (2004) estimated 55% and 65% nitrogen partitioning (assuming 5% nitrogen partitioning to respiratory mitochondrial enzymes) to

residual pools in temperature deciduous and evergreen trees, respectively, which overlaps with our residual pool range of 56–73% for tree PFTs. The large percentage of total leaf nitrogen in the residual pool is hypothesized to be used in structural (cell wall) proteins (6–14%; Takashima et al. 2004, Guan and Wen 2011), other nitrogen proteins (i.e., proteins not invested in photosynthesis, respiration, and structure; 15–25% after subtracting 5% mitochondrial proteins; Chapin et al. 1987, Takashima et al. 2004), free amino acids (2.5%; Chapin et al. 1987), lipids (3–4%; Chapin et al. 1986), nucleic acids (8.5–15%; Chapin et al. 1986, Chapin 1989, Evans and Seemann 1989), and CO_2 fixation proteins (other than Rubisco; 4%; Chapin et al. 1987). In addition, plants produce secondary compounds and metabolites (Bazzaz et al. 1987, Burns et al. 2002, Mithöfer and Boland 2012). Summing these estimates gives 39–65% nitrogen partitioned to the residual pool, which overlaps but is lower than our estimate of 56–73% possibly because our study includes nutrient limited tropical species having high residual pool sizes. Unlike previous studies that assessed nitrogen allocation for a few PFTs, our study reported substantial variation in leaf nitrogen allocation among different PFTs. The nitrogen allocations to different processes in this study are correlated to each other because the traits that are used to compute the nitrogen allocations are dependent on each other (e.g., V_{cmax} and J_{max}; Wullschleger 1993) and also because the nitrogen allocations are dependent on leaf nitrogen content.

In a related study, Xu et al. (2012) used a model fitted against a more limited number of site level V_{cmax} data to determine nitrogen allocations for needle-leaved evergreen trees (loblolly pine [*Pinus taeda*]), broadleaf deciduous trees (poplar [*Populus tremula*]), and herbaceous plants (Japanese plantain [*Plantago asiatica*]). However their study reported different magnitudes of nitrogen partitioning compared to our study due to differences in methodology and limited sample size in their study. For example, they reported low nitrogen partitioning to light capture (0.4–1.4%), and a higher fractional allocation to residual pools for the corresponding deciduous and evergreen PFTs compared to our estimates and corresponding lower fractional allocation to non-residual pools. These discrepancies are primarily due to their assumptions on the size and turnover time of the storage pool, which we did not include because it is difficult to parameterize. The storage pool used in their study is equivalent to the residual pool in our study (computed as the remaining leaf nitrogen not allocated to the photosynthetic and respiratory functions in our study) with the only difference that the residual pool in our study includes the structural nitrogen pool, which is a separate pool in their study. Finally, in predicting V_{cmax}, they assumed that J_{max}-limited-assimilation balances V_{cmax}-limited-assimilation, whereas we used estimates for J_{max} and V_{cmax} independently derived from observations.

Our study shows differences across PFTs (i.e., both increases and decreases) in fractional nitrogen allocation to Rubisco with changes in leaf nitrogen, in contrast to previous studies reporting either an increase or decrease in fractional nitrogen allocation to Rubisco with changes in leaf nitrogen. Our results show an increase in fractional leaf nitrogen allocation to Rubisco with increases in leaf nitrogen for crops, which supports the idea that crops have been selected to maximize productivity at the expense of other possible nitrogen investments, such as defense and structure. Our results are similar to the increase in fractional leaf nitrogen allocation to Rubisco with increases in leaf nitrogen reported by Evans (1989) for crops under carefully controlled conditions. In contrast, we found no change in fractional leaf nitrogen allocation to Rubisco with increase in leaf nitrogen for shrubs and broadleaf deciduous trees, suggesting that these PFTs allocate the same proportion of nitrogen to Rubisco irrespective of leaf nitrogen content. Furthermore, our results show a decrease in fractional leaf nitrogen allocation to Rubisco with increases in leaf nitrogen content for herbaceous plants, broadleaf evergreen trees, and needle-leaved evergreen trees. These latter results are corroborated by Coste et al. (2005), who found a decreasing relationship of fractional leaf nitrogen allocation to Rubisco with increases in leaf nitrogen for several tropical rain forest species. They attributed this decreasing relationship to differences in plant nitrogen use efficiencies and allocation strategies. This decreasing relationship in our study suggests that initial leaf nitrogen is invested in photosynthesis, with further nitrogen allocation to other functions. This decreasing fractional allocation frees up more nitrogen for the residual pools, which are associated with other functions, including reproduction, defense, structure, hormone production, storage, and reuse at a later time. The fractional leaf nitrogen allocation to residual pool correlates negatively with fractional leaf nitrogen allocation to photosynthetic and respiratory processes. In addition, the fractional leaf nitrogen allocation to electron transport, light absorption and Rubisco are positively correlated with each other.

The fractional leaf nitrogen allocation for different processes was obtained from the TRY database, when possible. When the database lacked a particular trait to derive the fractional nitrogen allocation, we integrated the data with a photosynthesis and respiration submodel. For instance, leaf nitrogen allocations to Rubisco and electron transport are obtained from measurement based values of V_{cmax} and J_{max} and well-characterized enzyme specific activity rates at standard temperature. Thus we have qualitatively higher confidence in these allocation estimates. This relatively higher confidence on the allocation for Rubisco is important because leaf nitrogen allocation to Rubisco is one of the most important parameters controlling photosynthesis in ecosystem models. Uncertainties in the derived relationships include those resulting from the assumption that Rubisco is fully active (Rogers 2014).

Nitrogen allocation to growth and maintenance respiration are obtained by integrating data and a respiration

sub-model. Because this process includes both models and direct measurements, we have lower confidence in the estimated nitrogen demands of respiration. However, the plant respiration models used in this study are effective, simple, and consistent with observations at multiple sites that plant respiration is a fixed fraction of gross productivity (Waring et al. 1998, Gifford 2003, Vicca et al. 2012). The remaining nitrogen not allocated to photosynthesis and respiration is attributed to the residual pool. Nitrogen allocation to the residual pool varies among different PFTs. For example, studies have found that nitrogen allocation for defense against biotic and abiotic stressors can vary between plants due to differences in the type, distribution, and amount of defense compounds (e.g., cyanogenic glucosides, glucosinolates, terpenoids, alkaloids, and phenolics) in plants (Bazzaz et al. 1987, Mithöfer and Boland 2012). There is substantial uncertainty in the literature, and no information in the TRY database, regarding how much nitrogen is allocated to individual functions accessing the residual pool, such as defense and hormone production. We therefore identify this lack of information as a critical need for ecosystem models, particularly since the residual nitrogen allocation is a large proportional investment in leaves for some PFTs, and since allocation of nitrogen to these currently undefined processes may be required to understand tradeoffs associated with different nitrogen allocation strategies.

Conclusions

This study integrated traits derived from observations with a photosynthesis and respiration sub-model to derive global patterns of leaf nitrogen allocation for photosynthesis (Rubisco, electron transport, light absorption), respiration (growth and maintenance), and a residual pool. Across PFTs, the greatest proportion of leaf nitrogen content is allocated to the residual pools, followed by photosynthesis and respiration. Leaf nitrogen allocation to light absorption, a major sub-process of photosynthesis, is strongly controlled by light availability. Based on analysis with the TRY data, tropical broadleaf evergreen trees have relatively low leaf nitrogen allocation to Rubisco. Our estimates are consistent with several previous studies but also different from some other studies due to differences in methodology and assumptions used in deriving nitrogen allocation estimates. Unlike previous studies, we integrate and infer nitrogen allocation estimates across multiple PFTs, and report substantial differences in nitrogen allocation across different PFTs.

Both V_{cmax} and J_{max} (two key parameters that influence photosynthesis in land models) increase with increases in leaf nitrogen for all PFTs. With increasing leaf nitrogen, the fractional leaf nitrogen allocation to Rubisco (i.e., V_{cmax}) increases for crops, remains unchanged for shrubs and broadleaf deciduous trees, and decreases for herbaceous plants, broadleaf evergreen trees, and needle-leaved evergreen trees. The increasing relationship suggests that crops invest as much nitrogen as possible for Rubisco to maximize productivity. In contrast, the decreasing relationship for the natural PFTs implies that as more nitrogen becomes available, a lower proportion is allocated for maintaining higher photosynthetic rates suggesting that if investment in photosynthesis was already optimized, the extra nitrogen would be invested for other functions.

We contend that representation of functional leaf nitrogen allocation in models is important for mechanistic understanding of global change impacts on terrestrial ecosystems, including from nitrogen deposition, changes to soil nitrogen mineralization, and CO_2 fertilization. Our results show systematic patterns that are an advancement for current models, but also show that a large fraction of leaf nitrogen is not explained by our approach, and that a better understanding of the non-photosynthetic and non-respiratory leaf nitrogen requirements, including structural, defensive, and supporting metabolic functions, may be required to create mechanistic models of leaf nitrogen allocation for natural ecosystems.

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Literature Cited

Ainsworth, E. A., and A. Rogers. 2007. The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. Plant, Cell & Environment 30:258–270.

Amthor, J. S. 2000. The McCree–de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. Annals of Botany 86:1–20.

Bazzaz, F. A., N. R. Chiariello, P. D. Coley, and L. F. Pitelka. 1987. Allocating resources to reproduction and defense. BioScience 37:58–67.

Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, M. Bustamante, S. Cinderby, E. Davidson, and F. Dentener. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20:30–59.

Burns, A. E., R. M. Gleadow, and I. E. Woodrow. 2002. Light alters the allocation of nitrogen to cyanogenic glycosides in *Eucalyptus cladocalyx*. Oecologia 133:288–294.

Chapin, F. S. 1989. The cost of tundra plant structures: evaluation of concepts and currencies. American Naturalist 133:1–19.

Chapin, F. S., A. J. Bloom, C. B. Field, and R. H. Waring. 1987. Plant responses to multiple environmental factors. BioScience 37:49–57.

Chapin, F. S., E.-D. Schulze, and H. A. Mooney. 1990. The ecology and economics of storage in plants. Annual Review of Ecology and Systematics 21:423–447.

Chapin, F., G. Shaver, and R. Kedrowski. 1986. Environmental controls over carbon, nitrogen and phosphorus fractions in *Eriophorum vaginatum* in Alaskan tussock tundra. The Journal of Ecology 74:167–195.

Chen, J., J. Liu, J. Cihlar, and M. Goulden. 1999. Daily canopy photosynthesis model through temporal and spatial scaling for remote sensing applications. Ecological Modelling 124:99–119.

Coste, S., J.-C. Roggy, P. Imbert, C. Born, D. Bonal, and E. Dreyer. 2005. Leaf photosynthetic traits of 14 tropical rain forest species in relation to leaf nitrogen concentration and shade tolerance. Tree Physiology 25:1127–1137.

Delagrange, S. 2011. Light-and seasonal-induced plasticity in leaf morphology, N partitioning and photosynthetic capacity of two temperate deciduous species. Environmental and Experimental Botany 70:1–10.

Domingues, T. F., J. A. Berry, L. A. Martinelli, J. P. Ometto, and J. R. Ehleringer. 2005. Parameterization of canopy structure and leaf-level gas exchange for an eastern Amazonian tropical rain forest (Tapajos National Forest, Para, Brazil). Earth Interactions 9:1–23.

Domingues, T. F., L. A. Martinelli, and J. R. Ehleringer. 2007. Ecophysiological traits of plant functional groups in forest and pasture ecosystems from eastern Amazonia, Brazil. Plant Ecology 193:101–112.

Dungan, R. J., D. Whitehead, and R. P. Duncan. 2003. Seasonal and temperature dependence of photosynthesis and respiration for two co-occurring broad-leaved tree species with contrasting leaf phenology. Tree Physiology 23:561–568.

Ehleringer, J. R., and C. B. Field. 1993. Scaling physiological processes: leaf to globe. Academic Press, San Diego, California, USA.

Ellsworth, D., and P. Reich. 1993. Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. Oecologia 96:169–178.

Ellsworth, D. S., P. B. Reich, E. S. Naumburg, G. W. Koch, M. E. Kubiske, and S. D. Smith. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Global Change Biology 10:2121–2138.

Evans, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9–19.

Evans, J., and H. Poorter. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant, Cell & Environment 24:755–767.

Evans, J. R., and J. R. Seemann. 1989. The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences, and control. Plant Biology 8:183–205.

Farquhar, G., S. V. von Caemmerer, and J. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149:78–90.

Field, C. 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. Oecologia 56:341–347.

Fisher, J., S. Sitch, Y. Malhi, R. Fisher, C. Huntingford, and S. Y. Tan. 2010. Carbon cost of plant nitrogen acquisition: a mechanistic, globally applicable model of plant nitrogen uptake, retranslocation, and fixation. Global Biogeochemical Cycles 24, GB1014, doi:10.1029/2009GB003621.

Galloway, J. N., and E. B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. AMBIO: A Journal of the Human Environment 31:64–71.

Ghimire, B., W. J. Riley, C. D. Koven, M. Mu, and J. T. Randerson. 2016. Representing leaf and root physiological traits in CLM improves global carbon and nitrogen cycling predictions. Journal of Advances in Modeling Earth Systems. doi:10.1002/2015MS000538.

Gifford, R. M. 2003. Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. Functional Plant Biology 30:171–186.

Grassi, G., and U. Bagnaresi. 2001. Foliar morphological and physiological plasticity in *Picea abies* and *Abies alba* saplings along a natural light gradient. Tree Physiology 21:959–967.

Grassi, G., E. Vicinelli, F. Ponti, L. Cantoni, and F. Magnani. 2005. Seasonal and interannual variability of photosynthetic capacity in relation to leaf nitrogen in a deciduous forest plantation in northern Italy. Tree Physiology 25:349–360.

Guan, L.-L., and D.-Z. Wen. 2011. More nitrogen partition in structural proteins and decreased photosynthetic nitrogen-use efficiency of *Pinus massoniana* under in situ polluted stress. Journal of Plant Research 124:663–673.

Han, Q., T. Kawasaki, S. Katahata, Y. Mukai, and Y. Chiba. 2003. Horizontal and vertical variations in photosynthetic capacity in a *Pinus densiflora* crown in relation to leaf nitrogen allocation and acclimation to irradiance. Tree Physiology 23:851–857.

Han, Q., T. Kawasaki, T. Nakano, and Y. Chiba. 2004. Spatial and seasonal variability of temperature responses of biochemical photosynthesis parameters and leaf nitrogen content within a *Pinus densiflora* crown. Tree Physiology 24:737–744.

Harley, P., R. Thomas, J. Reynolds, and B. Strain. 1992. Modelling photosynthesis of cotton grown in elevated CO2. Plant, Cell & Environment 15:271–282.

Hirose, T., and M. Werger. 1987. Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. Oecologia 72:520–526.

Houlton, B. Z., Y.-P. Wang, P. M. Vitousek, and C. B. Field. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454:327–330.

Kattge, J. 2002. Zur Bedeutung von Stickstoff fürden CO_2-Düngeeffekt. Dissertation. Justus-Liebig-Universität, Gieen, Germany.

Kattge, J., S. Diaz, S. Lavorel, I. Prentice, P. Leadley, G. Bönisch, E. Garnier, M. Westoby, P. B. Reich, and I. Wright. 2011. TRY–a global database of plant traits. Global Change Biology 17:2905–2935.

Kattge, J., and W. Knorr. 2007. Temperature acclimation in a biochemical model of photosynthesis: a reanalysis of data from 36 species. Plant, Cell & Environment 30:1176–1190.

Kattge, J., W. Knorr, T. Raddatz, and C. Wirth. 2009. Quantifying photosynthetic capacity and its relationship to leaf nitrogen content for global-scale terrestrial biosphere models. Global Change Biology 15:976–991.

Kumagai, T., T. Ichie, M. Yoshimura, M. Yamashita, T. Kenzo, T. M. Saitoh, M. Ohashi, M. Suzuki, T. Koike, and H. Komatsu. 2006. Modeling CO_2 exchange over a Bornean tropical rain forest using measured vertical and horizontal variations in leaf-level physiological parameters and leaf area densities. Journal of Geophysical Research—Atmospheres 111, D10107, doi:10.1029/2005JD006676.

Le Roux, X., S. Grand, E. Dreyer, and F.-A. Daudet. 1999. Parameterization and testing of a biochemically based photosynthesis model for walnut (*Juglans regia*) trees and seedlings. Tree Physiology 19:481–492.

Le Roux, X., A. Walcroft, F. Daudet, H. Sinoquet, M. Chaves, A. Rodrigues, and L. Osorio. 2001. Photosynthetic light acclimation in peach leaves: importance of changes in mass: area ratio, nitrogen concentration, and leaf nitrogen partitioning. Tree Physiology 21:377–386.

LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–379.

Luo, Y., B. Su, W. S. Currie, J. S. Dukes, A. Finzi, U. Hartwig, B. Hungate, R. E. McMurtrie, R. Oren, and W. J. Parton. 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. BioScience 54:731–739.

Makino, A., and B. Osmond. 1991. Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. Plant Physiology 96:355–362.

Malhi, Y., C. Doughty, and D. Galbraith. 2011. The allocation of ecosystem net primary productivity in tropical forests. Philosophical Transactions of the Royal Society B 366:3225–3245.

Matson, P., K. A. Lohse, and S. J. Hall. 2002. The globalization of nitrogen deposition: consequences for terrestrial ecosystems. AMBIO: A Journal of the Human Environment 31:113–119.

Medlyn, B. E. 1996. The optimal allocation of nitrogen within the C3 photosynthetic system at elevated CO_2. Functional Plant Biology 23:593–603.

Medlyn, B., F. W. Badeck, D. De Pury, C. Barton, M. Broadmeadow, R. Ceulemans, P. De Angelis, M. Forstreuter, M. Jach, and S. Kellomäki. 1999. Effects of elevated [CO_2] on photosynthesis in European forest species: a meta-analysis of model parameters. Plant, Cell & Environment 22:1475–1495.

Medlyn, B. E., and P. G. Jarvis. 1997. Integration of results from elevated CO2 experiments on European forest species: the ECOCRAFT project. Impacts of Global Change on Tree Physiology and Forest Ecosystems. Springer, Netherlands.

Meir, P., B. Kruijt, M. Broadmeadow, E. Barbosa, O. Kull, F. Carswell, A. Nobre, and P. Jarvis. 2002. Acclimation of photosynthetic capacity to irradiance in tree canopies in relation to leaf nitrogen concentration and leaf mass per unit area. Plant, Cell & Environment 25:343–357.

Meir, P., P. E. Levy, J. Grace, and P. G. Jarvis. 2007. Photosynthetic parameters from two contrasting woody vegetation types in West Africa. Plant Ecology 192:277–287.

Mithöfer, A., and W. Boland. 2012. Plant defense against herbivores: chemical aspects. Annual Review of Plant Biology 63: 431–450.

Moorcroft, P., G. Hurtt, and S. W. Pacala. 2001. A method for scaling vegetation dynamics: the ecosystem demography model (ED). Ecological Monographs 71:557–586.

Natali, S. M., E. A. Schuur, and R. L. Rubin. 2012. Increased plant productivity in Alaskan tundra as a result of experimental warming of soil and permafrost. Journal of Ecology 100:488–498.

Niinemets, Ü., O. Kull, and J. D. Tenhunen. 1998. An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. Tree Physiology 18:681–696.

Niinemets, Ü., and J. Tenhunen. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. Plant, Cell & Environment 20:845–866.

Oleson, K. W., et al. 2013. Technical description of version 4.5 of the Community Land Model (CLM). National Center for Atmospheric Research, Boulder, Colorado.

Onoda, Y., K. Hikosaka, and T. Hirose. 2004. Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. Functional Ecology 18:419–425.

Potter, C. S., J. T. Randerson, C. B. Field, P. A. Matson, P. M. Vitousek, H. A. Mooney, and S. A. Klooster. 1993. Terrestrial ecosystem production: a process model based on global satellite and surface data. Global Biogeochemical Cycles 7:811–841.

Reich, P. B. 2012. Key canopy traits drive forest productivity. Proceedings of the Royal Society B 279:2128–2134.

Reich, P. B., S. E. Hobbie, T. Lee, D. S. Ellsworth, J. B. West, D. Tilman, J. M. Knops, S. Naeem, and J. Trost. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO2. Nature 440:922–925.

Reich, P. B., J. Knops, D. Tilman, J. Craine, D. Ellsworth, M. Tjoelker, T. Lee, D. Wedin, S. Naeem, and D. Bahauddin. 2001. Plant diversity enhances ecosystem responses to elevated CO2 and nitrogen deposition. Nature 410:809–810.

Reich, P. B., and J. Oleksyn. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences USA 101:11001–11006.

Reich, P., M. Walters, and D. Ellsworth. 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. Ecological Monographs 62:365–392.

Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1997. From tropics to tundra: global convergence in plant functioning. Proceedings of the National Academy of Sciences USA 94:13730–13734.

Ripullone, F., G. Grassi, M. Lauteri, and M. Borghetti. 2003. Photosynthesis–nitrogen relationships: interpretation of different patterns between *Pseudotsuga menziesii* and *Populus*× *euroamericana* in a mini-stand experiment. Tree Physiology 23:137–144.

Rogers, A. 2014. The use and misuse of V_c, max in Earth System Models. Photosynthesis Research 119:15–29.

Rogers, A., E. A. Ainsworth, and A. D. Leakey. 2009. Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? Plant Physiology 151:1009–1016.

Ryan, M. G. 1991*a*. Effects of climate change on plant respiration. Ecological Applications 1:157–167.

Ryan, M. G. 1991*b*. A simple method for estimating gross carbon budgets for vegetation in forest ecosystems. Tree Physiology 9:255–266.

Schuur, E. A., K. G. Crummer, J. G. Vogel, and M. C. Mack. 2007. Plant species composition and productivity following permafrost thaw and thermokarst in Alaskan tundra. Ecosystems 10:280–292.

Sheffield, J., G. Goteti, and E. F. Wood. 2006. Development of a 50-year high-resolution global dataset of meteorological forcings for land surface modeling. Journal of Climate 19:3088–3111.

Takashima, T., K. Hikosaka, and T. Hirose. 2004. Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. Plant, Cell & Environment 27:1047–1054.

Tissue, D. T., K. L. Griffin, and J. T. Ball. 1999. Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO_2. Tree Physiology 19:221–228.

Tissue, D., K. Griffin, R. Thomas, and B. Strain. 1995. Effects of low and elevated CO2 on C3 and C4 annuals. Oecologia 101:21–28.

Vicca, S., S. Luyssaert, J. Penuelas, M. Campioli, F. Chapin, P. Ciais, A. Heinemeyer, P. Högberg, W. Kutsch, and B. Law. 2012. Fertile forests produce biomass more efficiently. Ecology Letters 15:520–526.

Vitousek, P. M., and H. Farrington. 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. Biogeochemistry 37:63–75.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13:87–115.

Vitousek, P. M., L. R. Walker, L. D. Whiteaker, and P. A. Matson. 1993. Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. Biogeochemistry 23:197–215.

Walcroft, A., X. Le Roux, A. Diaz-Espejo, N. Dones, and H. Sinoquet. 2002. Effects of crown development on leaf

irradiance, leaf morphology and photosynthetic capacity in a peach tree. Tree Physiology 22:929–938.

Walcroft, A., D. Whitehead, W. Silvester, and F. Kelliher. 1997. The response of photosynthetic model parameters to temperature and nitrogen concentration in *Pinus radiata* D. Don. Plant, Cell & Environment 20:1338–1348.

Waring, R., J. Landsberg, and M. Williams. 1998. Net primary production of forests: A constant fraction of gross primary production? Tree Physiology 18:129–134.

Whitehead, D., A. S. Walcroft, N. A. Scott, J. A. Townsend, C. M. Trotter, and G. N. Rogers. 2004. Characteristics of photosynthesis and stomatal conductance in the shrubland species mānuka (*Leptospermum scoparium*) and kānuka (*Kunzea ericoides*) for the estimation of annual canopy carbon uptake. Tree Physiology 24:795–804.

Williams, K., F. Percival, J. Merino, and H. Mooney. 1987. Estimation of tissue construction cost from heat of combustion and organic nitrogen content. Plant, Cell & Environment 10:725–734.

Wilson, K. B., D. D. Baldocchi, and P. J. Hanson. 2000. Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. Tree Physiology 20:565–578.

Wohlfahrt, G., M. Bahn, E. Haubner, I. Horak, W. Michaeler, K. Rottmar, U. Tappeiner, and A. Cernusca. 1999. Interspecific variation of the biochemical limitation to photosynthesis and related leaf traits of 30 species from mountain grassland ecosystems under different land use. Plant, Cell & Environment 22:1281–1296.

Wright, I. J., P. B. Reich, J. H. Cornelissen, D. S. Falster, P. K. Groom, K. Hikosaka, W. Lee, C. H. Lusk, Ü. Niinemets, and J. Oleksyn. 2005. Modulation of leaf economic traits and trait relationships by climate. Global Ecology and Biogeography 14:411–421.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. Cornelissen, and M. Diemer. 2004. The worldwide leaf economics spectrum. Nature 428:821–827.

Wullschleger, S. D. 1993. Biochemical limitations to carbon assimilation in C3 plants—A retrospective analysis of the A/Ci curves from 109 species. Journal of Experimental Botany 44:907–920.

Wullschleger, S., R. Norby, and C. Gunderson. 1992. Growth and maintenance respiration in leaves of *Liriodendron tulipifera* L. exposed to long-term carbon dioxide enrichment in the field. New Phytologist 121:515–523.

Xu, L., and D. D. Baldocchi. 2003. Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. Tree Physiology 23:865–877.

Xu, C., R. Fisher, S. D. Wullschleger, C. J. Wilson, M. Cai, and N. G. McDowell. 2012. Toward a mechanistic modeling of nitrogen limitation on vegetation dynamics. PLoS ONE 7: e37914.

Zaehle, S., and D. Dalmonech. 2011. Carbon–nitrogen interactions on land at global scales: current understanding in modelling climate biosphere feedbacks. Current Opinion in Environmental Sustainability 3:311–320.

Zaehle, S., and A. Friend. 2010. Carbon and nitrogen cycle dynamics in the O-CN land surface model: 1. Model description, site-scale evaluation, and sensitivity to parameter estimates. Global Biogeochemical Cycles 24, GB1005, doi:10.1029/2009GB003521.

Zaehle, S., B. E. Medlyn, M. G. De Kauwe, A. P. Walker, M. C. Dietze, T. Hickler, Y. Luo, Y. P. Wang, B. El-Masri, and P. Thornton. 2014. Evaluation of 11 terrestrial carbon–nitrogen cycle models against observations from two temperate Free-Air CO_2 enrichment studies. New Phytologist 202:803–822.

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