**Increasing whole-plant nitrogen demand reduces leaf nitrogen responses to soil nitrogen addition in grasslands**

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**Abstract**

Terrestrial carbon and nitrogen cycles are closely coupled. As such, the land surface components of Earth System Models are now beginning to include explicit nitrogen cycles and this has been shown to alter carbon cycling dynamics and, thus, climate feedbacks. An important aspect of this coupling within these models is the assumed positive correlations between soil nitrogen, leaf nitrogen per leaf area (*N*area), and photosynthetic capacity, which results in greater simulated leaf assimilation capacity in areas with more soil nitrogen. While these relationships have some empirical support, other studies have shown that *N*area and photosynthetic capacity are primarily determined by climate and that soil nitrogen availability, instead, leads to increased tissue development and/or storage. Here, we reconcile these differences using theory and data from a globally distributed experimental nitrogen addition network (Nutrient Network). We show that, across the network, soil nitrogen addition increases both *N*area and aboveground plant biomass. However, we find that soil nitrogen addition is a poor predictor of *N*area, which can be well modeled from leaf mass per leaf area and climate alone. Additionally, we find that the positive *N*area response to soil nitrogen decreases as the stimulation of biomass by soil nitrogen increases. These results reconcile discrepancies between past studies and shows that *N*area is the product of both soil nitrogen availability as well as whole-plant nitrogen demand. That is, in cases where plants use added soil nitrogen to grow new tissues, *N*area is primarily the product of climate and leaf structure. Whereas, a positive relationship between soil and leaf nitrogen is observed when added nitrogen is not used for growth. These dynamics will be important to include in the next generation of ESMs.

**Introduction**

Globally, carbon and nitrogen cycles are closely coupled. This coupling has a strong influence on carbon fluxes between the atmosphere and the Earth’s surface (Thornton et al., 2007). For instance, land plants rely on nitrogen to build photosynthetic enzymes (Evan 1989). Thus, nitrogen is an important regulator of carbon fluxes into terrestrial ecosystems, as indicated by Earth System Models (ESMs) that simulate reduced plant carbon assimilation when nitrogen constraints are considered (Wieder et al. 2015, Thomas et al., 2015, Thornton et al., 2007). Given ongoing addition of nitrogen to terrestrial ecosystems (Vitousek et al. 1997, Galloway et al. 2004, 2008), it is critical to understand how this will manifest itself in terrestrial ecosystems to reliably predict the rate and magnitude of future climate change.

ESMs typically assume a positive relationship between soil nitrogen availability, leaf nitrogen on an area basis (*N*area), and photosynthetic capacity. The positive correlation between *N*area and photosynthetic capacity is commonly observed (Walker et al., 2014, Kattge et al., 2009, Evan 1989) and is thought to be the result of the fact that photosynthetic enzymes are typically nitrogen-rich (Evans and Seeman 1989, Evans and Clarke 2019). However, the positive correlation between soil nitrogen availability and *N*area is not as straightforward. This is because plant nitrogen allocation is dynamic (Onoda et al., 2017) and is likely the product of both soil nitrogen availability and tissue or organ-specific plant nitrogen demand (Paillassa et al., 2020), which itself is environmentally dependent (Perkowski et al., in prep).

A few recent studies have highlighted the significantly positive relationship between soil nitrogen availability and *N*area (Firn et al., 2019, Li et al., 2020, Liang et al., 2020). These studies generally reason that this positive correlation stems from the fact that leaf photosynthesis is limited by nitrogen available to build nitrogen-rich proteins such as Ribulose-1,5-bisphosphate (Rubisco) that are involved in carboxylation. This generally follows previous conclusions from leaf-level analyses (Walker et al., 2014, Kattge et al., 2009). However, analyses on Rubisco carboxylation suggest that leaves are not carboxylation-limited and are instead well set up to maximize the utilization of available light in a given environment (Smith et al., 2019, Smith et al., 2020). So, an increase in leaf nitrogen to build Rubisco under nitrogen addition would be a wasteful process in the sense that the extra Rubsico would not increase photosynthesis unless it was accompanied by a similar increase in light energy. Nonetheless, a plant may choose to extra available nitrogen to build Rubisco as a means to maintain similar rates of photosynthesis at a lower stomatal conductance, effectively reducing nutrient use efficiency to increase water use efficiency (Wright et al., 2003). Global studies have found empirical support for this response in some contexts (Prentice et al., 2014, Paillassa et al., 2020).

Other studies have highlighted the importance of nitrogen demand for predicting *N*area (Dong et al., 2017, Onoda et al., 2017). Variations in demand is typically tied to variations in aboveground climatic conditions (Smith et al., 2020). Both ecophysiological theory and data (Smith et al., 2019, Dong et al., 2017) suggest that plant demand for nitrogen to build photosynthetic proteins decreases with temperature (Smith et al., 2020, Smith et al., 2018, Rogers et al., 2017, Hinojo-Hinojo, 2018, Ali et al., 2015, Smith et al., 2019, Dong et al., 2017, Paillassa et al., 2020) and CO2 (Smith et al., 2020, Ainsworth and Long, 2005, Ainsworth and Rogers, 2007, Leakey et al., 2009) and increases with light availability (Smith et al., 2019, Dong et al., 2017, Niinemets et al., 2015, Paillassa et al., 2020) and leaf-atmosphere vapor pressure deficit (Smith et al., 2019, Dong et al., 2017, Wang et al., 2017, Paillassa et al., 2020). In fact, previous data on Rubisco carboxylation capacity (Smith et al., 2019, Paillassa et al., 2020) and leaf nitrogen (Dong et al., 2017, Firn et al., 2019, Paillassa et al., 2020) suggest that this climate-driven changes in demand may be as, or even more important than soil nitrogen availability.

Plant ecophysiological theory (Wright et al., 2003, Franklin et al., 2020, Paillassa et al., 2020) provides a framework for reconciling the impact of soil nitrogen availability and plant nitrogen demand on *N*area. This theory suggests that a change in *N*area (∆*N*area) is the result of relative changes in nitrogen availability (∆*N*supply) and nitrogen demand (∆*N*demand):

∆*N*area ~ ∆*N*supply/∆*N*demand                                                                                                                (1)

where *N*supply is the nitrogen available for uptake and *N*demand is whole plant demand to build new tissues. Thus, an increase in *N*supply would increase *N*area as a means to increase water use efficiency only when there is a smaller accompanying increase in *N*demand (Figure 1 grey dashed line). If *N*demand changes in concert with *N*supply, we would expect no change in *N*area because all of the N would be allocated to build new tissues (Figure 1 black solid line). Different environmental contexts (e.g., canopy openness) may dictate how *N*demand varies with *N*supply and the resulting impact on *N*area.

Here, we use leaf-level and biomass data from a globally distributed grassland nutrient addition experiment, Nutrient Network (NutNet; Lind 2016), alongside ecophysiological theory to better understand the response of *N*area to nitrogen addition. Our aims were three-fold. Specifically, we aimed to (1) quantify the impact of soil nitrogen on *N*area, (2) separate soil nitrogen drivers of *N*area from climate drivers of *N*area, and (3) separate the impacts of nitrogen demand and nitrogen availability on *N*area. We hypothesized that both soil nitrogen availability and climate would have significant impacts on *N*area, but that the effect of soil nitrogen availability would be relatively weak due to the alternative ways in which plants can allocate available nitrogen. Following from this, we expected that *N*area responses would be weaker than leaf area index (LAI) responses because the optimal strategy for increasing productivity in grasslands would be to increase LAI rather than increase water use efficiency. Finally, we hypothesized that the *N*area response to soil nitrogen availability would be greatest in species that did not show a large increase in biomass, as these species likely were not exhibiting a concomitant increase in nitrogen demand.

**Methods**

* NutNet description
* NutNet datasets
* Analyses
  + Drivers of leaf Narea (mixed model)
    - Dependent variable: leaf Narea
    - Fixed effects: soil N \* soil P \* soil K + χ + temperature + PAR + LMA +Nfixer + photosynthetic pathway
    - Random terms: species + species:site + species:site:block
  + Predictability of leaf Narea (mixed model)
    - Dependent variable: leaf Narea
    - Fixed effects: soil N \* soil P \* soil K + predicted Nphoto + predicted Nstructure + Nfixer + photosynthetic pathway
    - Random terms: species + species:site + species:site:block
  + LAI response to soil N
    - Dependent variable: plot LAI
    - Fixed effects: soil N \* soil P \* soil K
    - Random terms: site + site:block
  + Supply/demand effects on leaf Narea (linear model)
    - Dependent variable: change in plot averaged leaf Narea in N addition plots
    - Fixed effects: change in plot LAI in N addition plots

**Results**

* Do these based on each question
  + Does soil N impact leaf Narea?
  + How does the impact of soil N on leaf Narea compare to other drivers, such as climate and LMA?
  + How does the leaf Narea response to soil N compare to the LAI response to soil N?
  + Does the impact of soil N on leaf Narea vary with leaf N demand, as indexed through the LAI response?

**Discussion**