

Comments from anonymous Referee #2

This study sets out to predict leaf nitrogen per unit area (N_{area}) through a combination of leaf mass per unit area (LMA), the ratio of leaf-internal to atmospheric CO₂ ($c_i:c_a$) and Rubisco activity. Although the study presents some interesting observations relating environmental variables to N_{area} and other leaf-scale traits, a major omission has been made by not showing explicitly how nitrogen per unit leaf mass (N_{mass}) varies in these observations. It is possible to infer some aspects of the relationships from the data presented, but it seems possible that a much simpler and perhaps stronger predictive relationship could be formulated around the simple fact that $N_{area} = LMA * N_{mass}$. This relationship is clear to the authors as they use it to calculate N_{area} itself from measurements of LMA and N_{mass} (p.5 line 24).

Response: We have carried out an analysis of $\ln N_{mass}$, parallel to our analysis of $\ln N_{area}$, and propose to present this in an Appendix. But since the relationship between $\ln N_{mass}$ and $\ln N_{area}$ can be expressed by $\ln N_{area} = \ln LMA + \ln N_{mass}$, the results are predictable: the partial relationships to variables other than $\ln LMA$ are unchanged, while the regression coefficient of N_{mass} with respect to $\ln LMA$ is reduced by 1. Because the coefficient of N_{area} with respect to $\ln LMA < 1$, the coefficient of N_{mass} with respect to $\ln LMA < 0$ (i.e. N_{mass} declines with LMA). But this relationship is neither simpler or stronger than our main analysis.

We also tried an analysis of N_{mass} omitting LMA as a predictor, but this resulted in a much poorer fit with several non-significant coefficients. We propose to add some explanation of these additional results in the discussion.

“We also performed a parallel regression using leaf nitrogen content per unit mass (N_{mass}) which showed, as expected, identical fitted coefficients for all predictors except LMA (Appendix B: Table B1 and Fig. B1). However, because the regression coefficient of $\ln N_{area}$ with respect to $\ln LMA < 1$, the regression coefficient of $\ln N_{mass}$

with respect to $\ln LMA < 0$, i.e. N_{mass} declines with increasing LMA, as has been widely reported. We also tried a regression of N_{mass} on the same set of predictors but without the inclusion of LMA; this yielded a much poorer fit and is not shown.”

The authors attempt to separate the LMA contribution to variation in Narea from a metabolic contribution, but they arrive at a summation of effects, one connected to structural variation which is tightly connected to LMA, and another metabolic component that is formulated as independent of LMA (p.2 lines 12-14, p.7 lines 4-6). My concern with this approach is that the metabolic component of Narea includes a dependence on LMA as well, since metabolic variation can be driven both by changes in the leaf tissue N concentration and by the number of layers of mesophyll cells and the thickness of each layer.

Response: We independently predict the structural and metabolic components of leaf N. The structural component of leaf N is assumed to be proportional to LMA, and this assumption is supported by an independent analysis of the relationship between cell-wall N and LMA (see p. 11, line 14). The metabolic component of leaf N is assumed to be proportional to V_{cmax} at a given temperature, which is predicted as a function of irradiance, leaf-internal CO₂ concentration (c_i) and temperature.

Now in reality, as the referee notes, V_{cmax} is not entirely independent of LMA, because leaves with high V_{cmax} require high LMA. But this means they require more structural N as well. Our multiple regression approach remains valid, even if LMA and V_{cmax} are partially correlated; the fact that we obtain independently significant regression coefficients indicates that both make separate contributions to determining N_{area} .

We propose to add a sentence to (a) recognize the partial dependence of LMA on V_{cmax} and (b) note how this is handled by multiple regressions in the discussion.

“These two predictors are not fully independent, because leaves with higher photosynthetic capacity tend to have higher LMA for structural reasons. But such leaves must have increased structural N as well. By showing independently significant

regression coefficients for modelled $N_{Rubisco}$ and LMA, the multiple regression approach establishes that successful prediction of N_{area} requires consideration of both components.”

Without explicitly showing how N_{mass} is related to the environmental factors explored here, it is not clear how the current study moves the field forward from the relationship suggested by Niinemets and Tenhunen (1997) between V_{cmax} and N_{area} .

Response: The proposed revision will include a demonstration of the partial relationships between N_{mass} and environmental variables, as mentioned above.

Niinemets and Tenhunen (1997) is an important reference for this research. Their focus was on explaining the observed vertical gradients of photosynthetic capacity and N_{area} within a tree canopy. Our focus is on predicting observed patterns in N_{area} across species and environments. Our success in doing so represents a significant advance on earlier work.

There is also a potential incongruency in the calculation of irradiance as a function of canopy leaf area, while asserting that the leaves measured were from the sunlit canopy. If truly sunlit leaves were used, then the relevant irradiance would be the top of canopy values. Perhaps this is just a matter of defining what sunlit leaves means for species that exist only in the understory of mixed species canopies. In any case, I am concerned that the irradiance used for sunlit leaves of the dominant trees in these relationships is not the correct one.

Response: Our terminology was wrong: we should have referred to ‘outer canopy’ leaves rather than ‘sunlit’ leaves, and we propose to amend this in the revision.

*“Mature **outer-canopy** leaves were sampled during the growing season using the AusPlots methodology (White et al. 2012).”*

By calculating a canopy-average irradiance, we represent the conditions likely to be experienced by species on average. This will indeed underestimate the irradiance experienced by the outer leaves of dominant trees or shrubs. It will also overestimate the irradiance experienced by plants at ground level. These errors presumably contribute to the scatter around the fitted relationship of N_{area} with irradiance. We add some words of explanation on this point in the method.

“In dense vegetation I_L will underestimate the PAR exposure of canopy dominants and overestimate the PAR exposure of understory species. However, the use of a canopy average in this way was a necessary approximation (because we did not have quantitative information about the canopy position of each species) and considered preferable to using I_0 , which will systematically overestimate PAR exposure for most species in a dense community.”

Appendix B: Partial responses of N_{mass} to environmental predictors

Table B1. Linear regression coefficients for $\ln N_{\text{mass}}$ (g g^{-1}). 100 as a function of $c_i:c_a$ (from $\delta^{13}\text{C}$), \ln (mean canopy PAR, I_L) ($\mu\text{mol m}^{-2} \text{s}^{-1}$), MAT ($^{\circ}\text{C}$), \ln LMA (g m^{-2}) and the factor ‘N-fixer’ at species level. Note N_{mass} was multiplied by 100 before log transformation.

	Estimated	Predicted	p	R^2
$c_i:c_a$	-0.611 ± 0.252	-0.615	<0.01	51%
$\ln I_L$	0.874 ± 0.096	1	<0.001	
MAT	-0.047 ± 0.007	-0.048	<0.001	
\ln LMA	-0.585 ± 0.036	n/a	<0.001	
‘N-fixer’	0.306 ± 0.041	n/a	<0.001	

Fig B1. Partial residual plots for the regression of $\ln N_{mass} (g g^{-1}) \cdot 100$ as a function of $c_i:c_a$ (from $\delta^{13}C$), \ln (mean canopy PAR, I_L) ($mmol m^{-2} s^{-1}$), MAT ($^{\circ}C$), \ln LMA ($g m^{-2}$) and the factor ‘N-fixer’ at species level. Note N_{mass} was multiplied by 100 before logarithmic transformation.

