nature portfolio

Peer Review File

Nitrogen deposition reveals global patterns in plant and animal stoichiometry

Corresponding Author: Dr Angelica Gonzalez

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
General Comments:
The manuscript presents an investigation into nitrogen (N) and phosphorus (P) stoichiometry and its implications for ecological and metabolic processes. While the topic is relevant and significant within the fields of ecological stoichiometry and biogeochemistry, there are several critical issues with this manuscript.

Major comments:

The title suggests a direct relationship between cellular metabolic processes and N/P ratios. However, it does not adequately reflect the complexity of the consideration of nutrient availability. Low N and P ratios associated with high N and P concentrations have been linked to metabolic constraints that primarily involve the levels of phosphorus-rich RNA, impacting protein synthesis. Addressing this complex relationship would enhance the clarity of the title.

The manuscript fails to encompass broader view including the roles of multiple elements, not just nitrogen and phosphorus. This oversight diminishes the robustness of the manuscript and its integration into the existing body of literature. On page 116, the manuscript limits its scope on stoichiometry to N and P while neglecting other significant elements involved in stoichiometric studies, such as carbon and potassium. Incorporating more comprehensive stoichiometric principles would strengthen the study.

The section on long-term acquired traits in evolutionary processes (p. 106) lacks clarity. The implications of these traits on N and P availability and utilization need to be considered more thoroughly.

The claim regarding protein synthesis being primarily limited by amino acid concentration rather than by P-rich RNA needs clearer justification (p. 143). More context or evidence supporting this assertion is requiered.

While the objectives stated on page 197 are commendable, they do not seem clearly connected to the results outlined in the manuscript. A stronger link between objectives and findings is needed.
The manuscript posits that if N remains unchanged while P levels increase, then the latitudinal change in N/P stoichiometry is driven by P; however, this relationship needs more robust exploration or justification (p. 210).

There is a lack of clarity in statements regarding the relationship between N content, availability, and temperature (p. 270). This section warrants a more thorough explanation of how these factors interrelate to aid reader comprehension.

The manuscript contains tautological statements, particularly concerning N availability, as it is influenced by various climatic and environmental factors (p. 349). These relationships warrant clarification with distinct examples.

Minor Comments:

The manuscript frequently shifts focus without making explicit connections between different sections and ideas. Improving the transitions and thematic connections is needed for a better flow and coherence.

Clarify the writing around p. 285) : not easy to understand.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
The manuscript by Gonzaléz provides an important, novel, and timely assessment of global patterns and the key factors influencing plant and animal stoichiometry. Understanding the factors that control stoichiometry in organisms is crucial for evaluating the impacts of anthropogenic changes on nutrient balances in the biosphere. While global patterns of plant stoichiometry have been widely studied, much less is known about animals, particularly in both terrestrial and aquatic ecosystems. This study leverages a novel database to provide a high level global estimates of animal stoichiometry. Furthermore, the research is grounded in a robust theoretical framework, enabling a comprehensive and detailed examination of these global patterns. The finding that nitrogen availability drives plant and animal stoichiometry is somewhat expected due to known plant-soil feedbacks, yet it provides valuable and novel knowledge at a global scale.

The manuscript along with its unique data set deserves publication and will potentially attract high scientific attention. However, while the statistical analysis and mechanistic interpretation are robust, there are two significant shortcomings that provide false or potentially misleading information and that prevent an acceptance: (1) an inadequate assessment of a key controlling factor—soil nutrient availability—and (2) insufficient documentation of plant element analysis and data use. These limitations potentially undermine the understanding of the factors driving organismal stoichiometry, thereby diminishing the impact of the manuscript's findings.

(1) Global nitrogen (N) availability has been mistakenly inferred from N deposition data, which does not necessarily equate to N availability in ecosystems. N deposition accounts for only a small fraction of total ecosystem N and in most ecosystems, deposited N becomes immobilized in the soil, with only a small portion remaining available to plants. The magnitude of this effect depends on the inherent soil N status (e.g., Nadelhofer et al., 1999, Nature; Du et al., 2020, Nature Geoscience). Consequently, using N deposition as a proxy for N availability is potentially misleading and introduces biases, particularly in regions with high N deposition such as China and Central Europe. This methodological flaw may also explain why the authors did not observe the anticipated relationship between (their presumed) N availability and latitude (as in other global studies). That said, the results do indicate that N deposition influences organismal stoichiometry. If N deposition is used as a variable, it should be explicitly referred to as "N deposition" and discussed in this context.
Similarly to N, total soil P, which the authors have used as a proxy for available P, may not accurately represent P availability. Phosphorus availability is influenced by additional factors such as soil pH and mineralogy (e.g., Augusto et al., 2017, Global Change Biology). Consequently, the lacking relation of organismal stoichiometry with the (apparent) soil P availability might at least partly be related to a misestimate. If total soil P is used, it should be explicitly labeled as "total P." However, there are existing datasets and models in the literature that provide more accurate estimates of global soil N and P availability and/or N-P limitation. For example, see Augusto et al. (2017, Global Change Biology) or Du et al. (2020, Nature Geoscience).

Since all relationships to stoichiometry depend on accurately estimated parameters—and given that the title emphasizes the importance of "N availability"—it is crucial to estimate nutrient availability as precisely as possible. Alternatively, the parameters should be labeled appropriately to reflect their true nature.

2. The findings on plant N and P contents are puzzling and contradict established knowledge. While the manuscript reports no relationship between plant N contents with latitude and temperature, nearly all previous studies show declining N contents in plant tissuies with increasing latitude, reflecting global patterns in soil N availability (e.g., Reich & Oleksyn, 2004, PNAS). While this could represent an interesting and novel observation, the discrepancy compared to previous studies may also stem from methodological issues such as inappropriate sampling (which is insufficiently described) and/or sampling from different land-use or vegetation types (also not detailed).
To address potential biases, the manuscript should clarify whether plant element content refers to N and P concentrations in total biomass or specific plant organs. This is also not clear from the data base. At a minimum, the main text section should specify that N and P contents were measured in foliage and shoots. However, also from the methods, it remains unclear how elements in trees were assessed and included in the data analysis, particularly since stems, with their low N and P concentrations, differ significantly from foliage, which has narrower ratios. If both were included, it would not be surprising that no clear relationships with latitude were detected. If only foliage has been used, this has to be written.
Additionally, N and P contents exhibit pronounced seasonality, and it is unclear from the text whether samples were collected exclusively during the growing season or included senescent stages, where nutrient contents are markedly lower. As sampling from different plant organs and at different seasons can profoundly impact the reported values and patterns, this distinction is crucial.
Therefore, it is imperative that the manuscript explicitly documents how plant N and P contents were quantified and ensures that these methods align with established standards to provide reliable and interpretable results.

Less crucial but still relevant are the following two shortcomings:
3. The spatial distribution of the sample set is puzzling, with seemingly very few terrestrial heterotrophs sampled outside of Europe. Based on my understanding of the database, it includes both autotroph and heterotroph samples collected at the

same locations. Please clarify whether this is the case. If not, how does the separation of sampling locations for autotrophs and heterotrophs impact the results?
4. Additionally, the units for nutrient availability are missing in all graphs. It is unclear whether the values are presented as molar or mass ratios of N/P. While this omission can be addressed quickly, it raises concerns about whether the central parameters are being handled with sufficient quantitative rigor.

Minor comments (party specifying the general comments)
L. 200 ff: Provide clear information about which animal groups are included or at least dominate in the database. Particularly puzzling is the treatment of plants, which possibly dominate the dataset of autotrophs. At L. 219, the authors speak from plants instead of autotrophs.
L. 201: Clearly write that soil N availability is represented by N deposition, while soil P availability is based on total P.
L. 209: The statement about trends is incorrect; generally, N in plants and microbes decreases with latitude. This seems to be a simple mistake, but the absence of a trend in autotrophic organisms remains puzzling. Given that plants (likely dominant among terrestrial autotrophs) typically show pronounced latitudinal patterns (e.g., Reich and Oleksyn, 2004, PNAS), it raises questions about which other groups or samples from various plant organs or from different land-uses in the dataset might obscure this pattern.
L. 219: The authors refer to “this decline in the N:P ratio of terrestrial plants,” using it synonymously with autotrophs. Please clarify whether “terrestrial plants” is intended to represent all autotrophs or just a subset.
L. 253: The discrepancy in environmental controls on plant N and P compared to previous studies is puzzling. Observed global patterns in plant nutrients are well-established and robust, which casts doubt on the conclusions of this study. One potential source of bias is the misestimation of N and P availability. Additionally, it is unclear which plant functional types and land-use categories were sampled. Factors such as fertilization or land use can override latitudinal patterns, further complicating interpretations. For these reasons, I remain unconvinced by the explanations provided by the authors.

Figure 1: provide units for the parameters analyzed. Specify temperature.
Figure 3/4: are the molar or mass ratios
Figure 4; extended Figure 3, 4: provide units of N availability on axis.
Extended Data Figure 3. Pearson correlation requires normally distributed data, but this does not seem to be the case.

(Remarks on code availability)

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors’ responses demonstrate a good effort to address the reviewers' concerns and improve the manuscript. However, some responses are more convincing and thorough than others. The authors need strengthening those that are less direct or that potentially bypass the core issue raised by the referees.

Regarding Reviewer #1 comments
Title Suggestion: It is true that similar titles exist, but the referee is highlighting a lack of specificity. Instead of only citing similar titles, you should briefly explain why a more specific title is undesirable in your context.
Broader Stoichiometric Perspective: Your response is good in acknowledging the need for a broader perspective and adding to the discussion. However, it doesn't fully address the referee's concern about the integration of a more comprehensive stoichiometric framework.
Could you discuss how including other elements would alter some of your findings? Could you provide more info on how you may incorporate the additional elements without a large data set?
Protein Synthesis Limitation: consider adding a statement of alternative hypothesis?
OK with the other responses to referee 1

Reviewer #2
More Self-Critical Reflection: You address the referee's concerns well but try to also demonstrate critical self-reflection on your own methodology.
Quantify limitations in the Data Set: Can you give a rough percentage estimate of missing data or the range of uncertainty for key variables? This would add to the transparency of your analysis.

So, in summary I would accept the paper, but only if there is ample space available. Otherwise, it could be transferred to a more specialized journal.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
In their revisions, the authors have successfully addressed the comments. The manuscript provides an important, novel and timely assessment of global patterns of plant and animal stoichiometry, making use of a large unique data base.

I have a few minor comments that might help to improve the manuscript.

With respect to nitrogen deposition, I would recommend presenting its use as a proxy for nitrogen availability and limitation early on, rather than hiding it. You may present it already in the methods section of the 'Nutrient-Dependent Physiology' part of the introduction (lines 172–182). I think one of the key findings requires more prominence and critical reflection.

L. 146 typo… instead of '.' it should read as ...., resulting in a
L. 244 “near the poles” reads irritating as no samples have been taken at the poles or nearby.
L. 365 In addition to leaching, low labile P and low P contents in foliage at low latitude, the high iron and aluminum oxide content in tropical soils bind phosphorus, rendering it unavailable to plants.
L. 374 Add 'soil properties' to the other environmental factors that modulate the effects of N deposition – they are likely to be central to controlling the supply of N through N mineralisation and retaining N deposition through immobilisation processes.

Overall, a more nuanced use of ‘driven’: I recommend using it only if there is a 'driving' mechanism, and not if other mechanisms 'cause' a certain pattern.
For example, at line 228, '...our results suggest that variation in N:P stoichiometry with latitude is primarily driven by changes in P content.'
In this case, it would be more appropriate to use 'is related to' or 'is caused by'.
This applies for more sentences

(Remarks on code availability)

Point-to-point responses to the comments of editors and reviewers
(Nature Communications manuscript NCOMMS-24-70829-T)

We sincerely thank the reviewers for their thoughtful and constructive feedback, which has significantly strengthened our manuscript.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

General Comments:
The manuscript presents an investigation into nitrogen (N) and phosphorus (P) stoichiometry and its implications for ecological and metabolic processes. While the topic is relevant and significant within the fields of ecological stoichiometry and biogeochemistry, there are several critical issues with this manuscript.

Major comments:

The title suggests a direct relationship between cellular metabolic processes and N/P ratios. However, it does not adequately reflect the complexity of the consideration of nutrient availability. Low N and P ratios associated with high N and P concentrations have been linked to metabolic constraints that primarily involve the levels of phosphorus-rich RNA, impacting protein synthesis. Addressing this complex relationship would enhance the clarity of the title.
Response: We appreciate the reviewer's thoughtful comment. However, we believe that the current title accurately reflects the central focus of our study. It highlights broad-scale patterns in stoichiometry across latitude, which are shaped by both physiological mechanisms and environmental drivers such as nutrient availability. Similar titles have been used in previous influential studies—e.g., Reich & Oleksyn, *"Global patterns of plant leaf N and P in relation to temperature and latitude"*; Martiny et al., *"Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter"*; and Yvon-Durocher, *"Temperature and the biogeography of algal stoichiometry"*. These works, like ours, emphasize geographic patterns while recognizing the underlying biochemical and ecological processes.

The manuscript fails to encompass broader view including the roles of multiple elements, not just nitrogen and phosphorus. This oversight diminishes the robustness of the manuscript and its integration into the existing body of literature. On page 116, the manuscript limits its scope on stoichiometry to N and P while neglecting other significant elements involved in stoichiometric studies, such as carbon and potassium. Incorporating more comprehensive stoichiometric principles would strengthen the study.
Response: We sincerely appreciate this insightful comment and fully agree that a broader stoichiometric perspective, including elements beyond nitrogen and phosphorus, is

important for advancing our understanding of biological and ecological processes. Elements such as carbon, potassium, and others undoubtedly play critical roles and are integral to a more holistic view of organismal stoichiometry.

However, the scope of our study was specifically designed to test hypotheses about large-scale variation in N and P content, and this focus was shaped by the current availability of global-scale data, which is heavily concentrated on N and P in animals. Unfortunately, comprehensive datasets for other elements remain limited, constraining our ability to integrate them into global analyses.

That said, we fully agree that expanding stoichiometric analyses to include additional elements is a valuable direction for future research. In response to the reviewer's suggestion, we have added this perspective to the discussion (L379–382): "Future stoichiometry research at large spatial scales will benefit from integrating organismal stoichiometry, including other key elements (e.g., carbon, potassium, calcium), with detailed information on abundance and resource stoichiometry data, enabling more robust predictions of the causes and consequences of variation in the stoichiometry of life."

The section on long-term acquired traits in evolutionary processes (p. 106) lacks clarity. The implications of these traits on N and P availability and utilization need to be considered more thoroughly.
Response: Thank you for this comment. However, we are somewhat unclear about the concern raised, as the section referenced (L106) does not explicitly mention evolutionary processes or long-term acquired traits. We would greatly appreciate further clarification to better understand which part of the manuscript the reviewer is referring to and what specific aspects require deeper consideration. This will help us address the concern more effectively and improve the clarity of the manuscript where needed.

The claim regarding protein synthesis being primarily limited by amino acid concentration rather than by P-rich RNA needs clearer justification (p. 143). More context or evidence supporting this assertion is required.
Response: We appreciate the reviewer's thoughtful comment and acknowledge the need to clarify our argument. Our intention was not to dismiss the role of RNA in protein synthesis but to emphasize that protein synthesis is ultimately constrained by the availability of amino acids, while RNA concentration primarily affects the rate of translation. This distinction is important in the context of the "hotter is better" hypothesis, which suggests that higher temperatures accelerate overall metabolic rates, including the synthesis of both N-rich proteins and P-rich RNA.
However, RNA synthesis has been shown to have a steeper temperature dependence than protein synthesis, leading to proportionally greater increases in P-rich RNA production at higher temperatures. This contributes to higher P content and lower N:P ratios in organisms from warmer climates. To address the reviewer's concern, we have revised the text to provide clearer context and supporting references (L137–146). The revised passage now reads " Each general mechanism (G) corresponds to one or two is rooted in specific

hypotheses. The first mechanism, *Temperature-Dependent Physiology,* GT°, the universal effects of temperature on metabolic processes. Two contrasting hypotheses emerge from this mechanism: the *hotter is better* and the alternative *metabolic cold adaptation* hypotheses. The *hotter is better* hypothesis posits that organisms in warm, low-latitude environments exhibit accelerated metabolic rates and, consequently, increased synthesis of N- rich proteins and P-rich RNA molecules. This enhanced biosynthetic activity leads to elevated N and P contents in organisms inhabiting warmer climates. Notably, because RNA synthesis is more temperature-sensitive than protein synthesis, P content is expected to increase more steeply than N content. Resulting in a lower N:P ratio in warmer, low-latitude environments22,23"

While the objectives stated on page 197 are commendable, they do not seem clearly connected to the results outlined in the manuscript. A stronger link between objectives and findings is needed.

Response: Thank you for this helpful suggestion. To improve the clarity and cohesion of the manuscript, we have strengthened the connection between the stated objectives and the results by explicitly naming the hypotheses (INTRA vs. INTER) in the introduction (L193–201). We now refer to these named hypotheses consistently throughout the results and discussion sections (e.g., L309-336), which makes it easier for readers to follow how the findings relate to the initial research objectives.

The manuscript posits that if N remains unchanged while P levels increase, then the latitudinal change in N/P stoichiometry is driven by P; however, this relationship needs more robust exploration or justification (p. 210).

Response: We appreciate the reviewer's comment regarding the need for a clearer justification of how changes in N:P stoichiometry are attributed to variation in phosphorus content. Our analysis indicates that nitrogen content does not vary significantly with latitude, whereas phosphorus content exhibits a clear positive trend. This pattern suggests that latitudinal shifts in N:P ratios are primarily driven by changes in P content rather than N. To clarify this reasoning, we have revised the relevant section to more explicitly link the patterns in elemental content with the observed stoichiometric shifts. The revised text now reads (L222–247): "Our analyses revealed that neither the N and P content of heterotrophs nor the N content of autotrophs exhibits latitudinal trends across terrestrial and freshwater realms (Fig. 2, Supplementary Fig. 2, and Supplementary Tables 1 and 2). These findings contrast with previous studies suggesting a significant, albeit weak, increase in the N content of plants11,12,51 and microbes52,53 at high latitudes. In line with earlier studies, however, we observed a significant increase in P content with latitude in freshwater and terrestrial autotrophs11,12,51and terrestrial organisms across trophic groups (Fig. 2A,B, and Supplementary Table 1). Given the absence of a latitudinal trend in N content alongside a clear increase in P content, our results suggest that variation in N:P stoichiometry with latitude is primarily driven by changes in P content. Notably, these patterns were largely driven by terrestrial autotrophs, predominantly composed of plants see Methods section), which exhibited, on average, a 173% increase in P content (back- transformed scale) from 0° to 80° absolute latitude (Fig. 2C and Supplementary

Table 1). Consistent with our predictions, terrestrial organisms exhibited a decline in N:P with increasing latitude (Fig. 3A and Supplementary Table 1), primarily driven by an increase in the P content of autotrophs (Fig. 2A, Fig. 3B and Supplementary Table 1). This latitudinal decrease in the N:P ratio of terrestrial autotrophs, also reported in previous studies11,12,51, reflects a steeper and significant increase in P content (slope coefficient = 1.3e-02) relative to a smaller, non- significant increase in N content (slope coefficient = 4.0e-03; Supplementary Table 1). The observed decline in N:P is likely attributable to the greater environmental sensitivity of P-rich hypothesized proposed mechanisms (temperature, radiation, and nutrients). Supporting the role of nutrient availability, the predicted mean N:P for terrestrial autotrophs declined markedly from 41.7 (39.2–44.5, 95% CI) near the equator to 15.3 (13.6–17.1, 95% CI) near the poles. An N:P above 20 has been proposed to indicate strong P limitation for plant growth and biomass production56 , suggesting that terrestrial autotrophs are more likely to experience P-limitation lower-latitude environments40."

There is a lack of clarity in statements regarding the relationship between N content, availability, and temperature (p. 270). This section warrants a more thorough explanation of how these factors interrelate to aid reader comprehension.

Response: We thank the reviewer for highlighting the need to clarify the relationships among nitrogen content, nitrogen availability, and temperature. To improve reader comprehension, we have revised this section to more clearly explain how these factors interrelate. Specifically, while our primary analyses did not detect a significant direct effect of temperature or radiation on organismal N content, we acknowledge that such effects may emerge through interactions with other environmental drivers.

To explore this, we tested whether temperature and labile phosphorus modulate the relationship between organismal N content and N deposition. Our results revealed that these interactions significantly strengthened the relationship (Extended Data Table 4), but contributed only marginally to model performance—accounting for an additional ~1% of the explained variance (Extended Data Table 2). This suggests that while interactions between temperature and nutrient availability do shape stoichiometric patterns, their overall contribution remains limited relative to the dominant effect of nitrogen deposition. We have revised the manuscript to enhance clarity and better integrate these findings into the broader discussion of environmental controls on stoichiometry. The revised section now reads (L295–307):

"To better understand how environmental factors influence organismal N content, it is important to consider both direct and interactive effects of temperature, nutrient availability, and other abiotic conditions. While our primary analyses did not detect a significant direct effect of temperature or radiation on organismal N content, these factors may still exert influence through interactions with nutrient availability62 . To test this possibility, we examined whether temperature and labile P together modulate the relationship between organismal N content and N deposition. Our results showed that these interactions significantly enhanced the relationship (Supplementary Table 4) yet accounted for only an additional 1% of the variance in organismal N content (Supplementary Table 2). This indicates that while energy-resource interactions do influence organismal stoichiometry, their combined effects are modest compared to the direct influence of N deposition. These findings are consistent with previous studies highlighting the context-dependent effects of environmental drivers on organismal elemental content67,68 while emphasizing the dominant role of N deposition in shaping global stoichiometric patterns."

The manuscript contains tautological statements, particularly concerning N availability, as it is influenced by various climatic and environmental factors (p. 349). These relationships warrant clarification with distinct examples.
Response: We appreciate the reviewer's feedback and acknowledge the need to clarify the role of nitrogen deposition and to avoid tautological statements. In response, we have revised the text to more clearly distinguish between nitrogen deposition as a proximate driver of stoichiometric patterns and the broader environmental and climatic processes that influence nitrogen availability. Specifically, we now describe how factors such as precipitation-driven leaching, temperature-mediated decomposition, and biological nitrogen fixation contribute to spatial variation in N availability. This revision ensures that the conclusions are more precise and avoid circular reasoning. The revised section (L371–382) now reads: "In conclusion, our findings underscore the role of N deposition as a key driver of global variation in the elemental content of plants and animals, with latitude, temperature, radiation, and labile P exerting limited or inconsistent effects. While N deposition itself is modulated by climatic and other environmental factors—including precipitation-driven leaching, temperature-mediated decomposition rates, and biological nitrogen fixation— its direct influence on organismal stoichiometry appears dominant. These patterns highlight the potential for human-driven shifts in nutrient inputs and climate to influence the elemental content of living organisms and multiple dimensions of biodiversity, including taxonomic, functional, and genetic diversity. Future stoichiometry research at large spatial scales will benefit from integrating organismal stoichiometry, including other key elements (e.g., carbon, potassium, calcium), with detailed information on abundance and resource stoichiometry data, enabling more robust predictions of the causes and consequences of variation in the stoichiometry of life."

Minor Comments:

The manuscript frequently shifts focus without making explicit connections between different sections and ideas. Improving the transitions and thematic connections is needed for better flow and coherence.

Clarify the writing around p. 285) : not easy to understand.
Response: We thank the reviewer for highlighting the need to improve clarity in this section. To enhance readability and ensure that the relationships between species-level nitrogen content, intraspecific variation, and environmental nitrogen deposition are more clearly articulated, we have revised the text accordingly. The updated version explicitly describes how species exposed to broader gradients of nitrogen deposition tend to show

greater intraspecific variation in N content, which may, in turn, increase their ecological tolerance and performance. The revised passage now reads (L316–325): "A more targeted analysis along the N deposition gradient revealed a significant positive relationship between environmental N deposition and the range of species-level N content (Supplementary Fig. 3). In support of our hypothesis *INTRA*, our results suggest that species experiencing broader environmental gradients in N deposition tend to exhibit greater intraspecific variation in N content—potentially enhancing their ecological tolerance and performance, as reported for other functional groups69. Given the established links between organismal stoichiometry, ecological performance, and evolutionary processes4,37, understanding the scope of these responses is essential for predicting the impacts of global environmental change—particularly those arising from nutrient enrichment37."

Reviewer #2 (Remarks to the Author):

The manuscript by Gonzaléz provides an important, novel, and timely assessment of global patterns and the key factors influencing plant and animal stoichiometry. Understanding the factors that control stoichiometry in organisms is crucial for evaluating the impacts of anthropogenic changes on nutrient balances in the biosphere. While global patterns of plant stoichiometry have been widely studied, much less is known about animals, particularly in both terrestrial and aquatic ecosystems. This study leverages a novel database to provide a high level global estimates of animal stoichiometry. Furthermore, the research is grounded in a robust theoretical framework, enabling a comprehensive and detailed examination of these global patterns. The finding that nitrogen availability drives plant and animal stoichiometry is somewhat expected due to known plant-soil feedbacks, yet it provides valuable and novel knowledge at a global scale.
Response: thank you**.**

The manuscript along with its unique data set deserves publication and will potentially attract high scientific attention. However, while the statistical analysis and mechanistic interpretation are robust, there are two significant shortcomings that provide false or potentially misleading information and that prevent an acceptance: (1) an inadequate assessment of a key controlling factor—soil nutrient availability—and (2) insufficient documentation of plant element analysis and data use. These limitations potentially undermine the understanding of the factors driving organismal stoichiometry, thereby diminishing the impact of the manuscript's findings.

(1) Global nitrogen (N) availability has been mistakenly inferred from N deposition data, which does not necessarily equate to N availability in ecosystems. N deposition accounts for only a small fraction of total ecosystem N and in most ecosystems, deposited N becomes immobilized in the soil, with only a small portion remaining available to plants. The magnitude of this effect depends on the inherent soil N status (e.g., Nadelhofer et al., 1999, Nature; Du et al., 2020, Nature Geoscience). Consequently, using N deposition as a

proxy for N availability is potentially misleading and introduces biases, particularly in regions with high N deposition such as China and Central Europe. This methodological flaw may also explain why the authors did not observe the anticipated relationship between (their presumed) N availability and latitude (as in other global studies). That said, the results do indicate that N deposition influences organismal stoichiometry. If N deposition is used as a variable, it should be explicitly referred to as "N deposition" and discussed in this context.

Response: We thank the reviewer for these important comments. We fully acknowledge the distinction between nitrogen deposition and nitrogen availability and agree that conflating the two can be misleading. Our intention was not to equate nitrogen deposition directly with ecosystem nitrogen availability but to use it as a proxy in the absence of a more comprehensive, standardized global dataset. To address this concern, we have made the following changes:

- We now explicitly state that nitrogen deposition is used as a proxy for nitrogen availability (L444-446) and have replaced all mentions of “N availability” with “N deposition” in the main text and figures where appropriate.
- We acknowledge the limitations of this proxy, especially in regions where N deposition may not reflect plant-available N due to soil buffering or retention effects (L452–461). “We acknowledge that those proxies might not be the best at representing nutrient availability, especially in regions with high N deposition rates (i.e., influencing N availability) or with contrasting soil mineralogy, pH, and microbial activity (i.e., influencing P availability). However, these proxies are, to our knowledge, the best data available given the spatial extent and diversity of terrestrial (e.g., grasslands, forests, shrublands) and freshwater (streams, ponds, lakes, rivers, phytotelmata) ecosystems included in our analyses.”

While we agree that N deposition reflects only part of the N cycle, it remains a widely used and ecologically relevant indicator in global-scale analyses. Numerous studies support its use as a driver of ecosystem nitrogen dynamics and biological responses, including:

- *Barton et al. (2018)*: “Nitrogen deposition significantly increases nitrogen availability in forest soils… often favoring nitrogen-tolerant species.”
- *Clark et al. (2016)*: “Nitrogen deposition enhances nitrogen availability in forest soils, often leading to increased soil nitrogen pools and changes in nutrient dynamics.”
- *Averill & Waring (2018)*: “Nitrogen deposition is a key driver of increased nitrogen availability in soils… influencing productivity and nutrient cycling.”
- *Barker et al. (2019)*: “Nitrogen deposition directly influences nitrogen availability in freshwater systems, contributing to nutrient loading and ecological change.”

Given the lack of global, spatially explicit measurements of soil N availability, we believe nitrogen deposition remains an appropriate and useful—though imperfect—proxy for large-scale comparative studies.

Regarding the datasets mentioned by the reviewer (Du et al., 2020; Augusto et al., 2017), we note that while valuable, they are based on nutrient addition experiments with limited spatial coverage (e.g., 106 and 53 sites), or are focused on plant response databases. These sources are informative but do not provide the comprehensive spatial coverage needed for global organismal stoichiometry analyses.

Similarly to N, total soil P, which the authors have used as a proxy for available P, may not accurately represent P availability. Phosphorus availability is influenced by additional factors such as soil pH and mineralogy (e.g., Augusto et al., 2017, Global Change Biology). Consequently, the lacking relation of organismal stoichiometry with the (apparent) soil P availability might at least partly be related to a misestimate. If total soil P is used, it should be explicitly labeled as "total P." However, there are existing datasets and models in the literature that provide more accurate estimates of global soil N and P availability and/or N-P limitation. For example, see Augusto et al. (2017, Global Change Biology) or Du et al. (2020, Nature Geoscience).

Response: We also appreciate the reviewer's point about the use of total or labile soil phosphorus as a proxy for P availability. We fully agree that bioavailable P is strongly modulated by soil pH, mineralogy, and microbial activity (e.g., Augusto et al., 2017). However, in the absence of a universally available and consistent global dataset on truly bioavailable P, labile P remains one of the most widely used proxies for comparative purposes.

To clarify this point:

- We have revised the text to explicitly label this variable as "labile P" throughout.
- We now acknowledge the limitations of using labile P as a proxy for availability and have incorporated this discussion into the revised manuscript (L452–461).

Since all relationships to stoichiometry depend on accurately estimated parameters—and given that the title emphasizes the importance of "N availability"—it is crucial to estimate nutrient availability as precisely as possible. Alternatively, the parameters should be labeled appropriately to reflect their true nature.

Response: We fully agree with the reviewer that precise terminology is essential, particularly when the interpretation of stoichiometric patterns depends on accurately characterizing nutrient availability. As discussed above, the lack of comprehensive, standardized global datasets for bioavailable nitrogen (N) and phosphorus (P) presents a significant challenge to directly estimating nutrient availability across ecosystems.

To ensure clarity and accuracy, we have revised the manuscript to explicitly refer to the environmental variables used in our analyses as "N deposition" and "labile P," rather than as proxies for nutrient availability. These terms are now consistently applied throughout the text and figures. In addition, we have expanded our discussion to acknowledge the limitations of these variables and to clarify how they may influence our interpretation of nutrient-stoichiometry relationships.

We appreciate the reviewer's attention to this important point and have taken care to enhance the rigor and transparency of the manuscript through these revisions.

2. The findings on plant N and P contents are puzzling and contradict established knowledge. While the manuscript reports no relationship between plant N contents with latitude and temperature, nearly all previous studies show declining N contents in plant

tissues with increasing latitude, reflecting global patterns in soil N availability (e.g., Reich & Oleksyn, 2004, PNAS). While this could represent an interesting and novel observation, the discrepancy compared to previous studies may also stem from methodological issues such as inappropriate sampling (which is insufficiently described) and/or sampling from different land-use or vegetation types (also not detailed).

Response: We thank the reviewer for this thoughtful comment. We agree that our findings on plant N content may initially appear to contradict earlier studies, such as Reich & Oleksyn (2004), which reported a weak but statistically significant decline in plant N content with increasing latitude. However, we believe that this discrepancy reflects both true biological variability and differences in methodological approaches—points we have now clarified more explicitly in the revised manuscript.

To address potential confusion, we highlight the following:

1. **Our findings align with earlier studies** in key respects. For example, consistent with Reich & Oleksyn (2004) and Borer et al. (2013), we found:
    - A significant increase in plant (autotroph) P content with absolute latitude (Fig. 2).
    - A significant latitudinal decline in N:P ratio in autotrophs (Fig. 3), primarily driven by changes in P content.
2. **We also identified novel patterns**, such as a strong positive relationship between N deposition and both N content and N:P ratios in autotrophs (Fig. 4). This relationship was not tested in Reich & Oleksyn (2004) or Borer et al. (2013), and highlights the influence of human-driven nutrient inputs on global stoichiometric patterns.
3. **Our analyses build on prior work** by incorporating:
    - The original Reich & Oleksyn dataset,
    - Additional data sources covering a wider range of ecosystems,
    - More robust statistical approaches, including permutation testing, mixed-effects models, and logit transformations.
4. **Differences in findings—particularly regarding plant N content—may reflect context-dependent or localized effects**, rather than inconsistencies or methodological flaws. Notably, the R^2 values for the latitude–N content relationships in earlier studies were low (e.g., R^2 = 0.04 in Reich & Oleksyn, 2004; R^2 = 0.05 in Borer et al., 2013), suggesting that these patterns, while statistically significant, explain only a small portion of the variance.
5. **We also detect temperature effects**, though primarily as interactions with other variables (see section beginning L278), indicating that climate influences may be more complex and conditional than previously assumed.

To improve clarity, we revised the manuscript in several places to more clearly state these agreements and discrepancies:

- L224: "These findings contrast with previous studies suggesting a significant, albeit weak, increase in the N content of plants [...] at high latitudes."
- L226: "In line with earlier studies, however, we observed a significant increase in P content with latitude in freshwater and terrestrial autotrophs[3],[10],[38] [...]"

- L236: "This latitudinal decrease in the N:P ratio of terrestrial autotrophs, also reported in previous studies[3],[10],[38] [...]"
- L273: "These results challenge previously reported effects of temperature and radiation on the elemental content of plants[3],[10] and microbes[4],[40] [...]"
- L278: "The consistent latitudinal patterns in plant P content and N:P ratio—both in our study and prior work[3],[10],[38]—point toward underlying global-scale mechanisms shaping autotroph stoichiometry. In contrast, the lack of consistent latitudinal trends in plant N content suggests that local or context-specific factors, such as soil nutrient status, land use, or fertilization history, may blur broader biogeographical patterns."

We hope these clarifications and revisions strengthen the manuscript and address the reviewer's concerns.

To address potential biases, the manuscript should clarify whether plant element content refers to N and P concentrations in total biomass or specific plant organs. This is also not clear from the data base. At a minimum, the main text section should specify that N and P contents were measured in foliage and shoots. However, also from the methods, it remains unclear how elements in trees were assessed and included in the data analysis, particularly since stems, with their low N and P concentrations, differ significantly from foliage, which has narrower ratios. If both were included, it would not be surprising that no clear relationships with latitude were detected. If only foliage has been used, this has to be written.
Response: We thank the reviewer for this important observation. We confirm that our plant data are based exclusively on leaf nitrogen and phosphorus content, which is standard in stoichiometric studies due to the high metabolic activity and ecological relevance of foliage. Stems and other plant organs, which differ markedly in nutrient concentrations and stoichiometric ratios, were not included in our dataset.
To eliminate any ambiguity, we have now explicitly stated in the Methods section that only (fresh not senescent) leaf N and P data were used (L388). In addition, we have referenced the StoichLife data paper, which provides further details on data sources and curation methods (L386). This clarification ensures transparency in our methodology and supports an accurate interpretation of our findings.

Additionally, N and P contents exhibit pronounced seasonality, and it is unclear from the text whether samples were collected exclusively during the growing season or included senescent stages, where nutrient contents are markedly lower. As sampling from different plant organs and at different seasons can profoundly impact the reported values and patterns, this distinction is crucial.
Response: We appreciate this important comment. As noted earlier, all plant samples included in our analysis were derived from leaf tissue, while animal samples were analyzed as whole-body content. We fully acknowledge the potential influence of seasonal variation on leaf nutrient content, particularly between peak growing season and senescent stages.

Most of the original studies contributing to the StoichLife dataset did not consistently report the exact timing of leaf sampling. However, to the best of our knowledge—and based on data screening procedures—we can confirm that senescent leaves were excluded, helping to minimize variability associated with late-season nutrient resorption. While the dataset may include some natural variation in sampling time within the growing season, we believe this variability is minor compared to global patterns and does not obscure the main trends. We have now clarified these points in the Methods section of the manuscript to ensure transparency and address the reviewer's concern.

Therefore, it is imperative that the manuscript explicitly documents how plant N and P contents were quantified and ensures that these methods align with established standards to provide reliable and interpretable results.
Response: We appreciate the reviewer's concern regarding the quantification of plant nitrogen (N) and phosphorus (P) contents. As our dataset is based on the StoichLife database, which compiles both published and unpublished data, the methods used to determine N and P concentrations are those originally reported by the contributing studies. For all unpublished data, detailed methodological descriptions are available in the associated StoichLife data paper, which ensures transparency and traceability. Importantly, the analytical methods used in these sources adhere to established standards widely accepted in plant and animal stoichiometry research (L391–394). We have clarified this in the manuscript to ensure that the provenance and reliability of the data are communicated.

Less crucial but still relevant are the following two shortcomings:
3. The spatial distribution of the sample set is puzzling, with seemingly very few terrestrial heterotrophs sampled outside of Europe. Based on my understanding of the database, it includes both autotroph and heterotroph samples collected at the same locations. Please clarify whether this is the case. If not, how does the separation of sampling locations for autotrophs and heterotrophs impact the results?
Response: We appreciate the reviewer's attention to the spatial distribution of our dataset. To clarify, the autotroph (plant) and heterotroph (animal) data were compiled independently and were not systematically collected from the same locations. These datasets originate from separate studies conducted by different research groups and were integrated into the StoichLife database for large-scale comparative analyses.
We acknowledge that terrestrial heterotroph samples are disproportionately concentrated in Europe. This pattern reflects the geographic bias in data availability rather than an intentional sampling design. While this limits direct comparisons between autotrophs and heterotrophs at the same sites, our analytical framework focuses on global-scale patterns rather than local co-occurrence.
To enhance transparency, we have added a statement to the manuscript clarifying this point (L397–398):
"The autotroph and heterotroph data were compiled from independent studies, and were not necessarily collected at the same locations."

4. Additionally, the units for nutrient availability are missing in all graphs. It is unclear whether the values are presented as molar or mass ratios of N/P. While this omission can be addressed quickly, it raises concerns about whether the central parameters are being handled with sufficient quantitative rigor.
Response: We thank the reviewer for pointing out this oversight. We have now added the appropriate units to all figures to ensure clarity and consistency. Specifically, nitrogen and phosphorus contents are expressed as percent of dry mass, and N:P ratios are presented as molar ratios, in line with standard practice in stoichiometric studies. We appreciate the opportunity to correct this and improve the quantitative clarity of the manuscript.

Minor comments (party specifying the general comments)
L. 200 ff: Provide clear information about which animal groups are included or at least dominate in the database. Particularly puzzling is the treatment of plants, which possibly dominate the dataset of autotrophs. At L. 219, the authors speak from plants instead of autotrophs.
Response: Thank you for this helpful comment. We agree that clearer information on the taxonomic composition of autotrophs and heterotrophs is important for interpretation. In addition to referencing the StoichLife database, we have now included a summary of the dominant taxa represented in each group to clarify this aspect of the dataset.
The following text has been added to the Methods section (L425–432):
'Terrestrial autotrophs are dominated by three main classes—Magnoliopsida (76%), Liliopsida (13%), and Pinopsida (6%)—while aquatic autotrophs are mainly represented by, Liliopsida (44%), Ulvophyceae (21%), and Florideophyceae (19%; in percent of all terrestrial or aquatic autotrophs, respectively). In turn, the three main classes of terrestrial heterotrophs are Insecta (32%), Arachnida (27%), and Collembola (17%) and aquatic heterotrophs are represented by Insecta (42%), Actinopterygii (27%) and Anthozoa (8%; in percent of all terrestrial or aquatic heterotrophs, respectively).'

L. 201: Clearly write that soil N availability is represented by N deposition, while soil P availability is based on total P.
Response: Thank you for highlighting this important point. We have now explicitly stated throughout the manuscript and figure captions that soil nitrogen (N) availability is represented by nitrogen deposition, and that soil phosphorus (P) availability is represented by labile P. These clarifications have been incorporated to ensure consistency, transparency, and accuracy in how we describe the environmental variables used in our analyses.

L. 209: The statement about trends is incorrect; generally, N in plants and microbes decreases with latitude. This seems to be a simple mistake, but the absence of a trend in autotrophic organisms remains puzzling. Given that plants (likely dominant among terrestrial autotrophs) typically show pronounced latitudinal patterns (e.g., Reich and Oleksyn, 2004, PNAS), it raises questions about which other groups or samples from various plant organs or from different land-uses in the dataset might obscure this pattern.

L. 219: The authors refer to "this decline in the N:P ratio of terrestrial plants," using it synonymously with autotrophs. Please clarify whether "terrestrial plants" is intended to represent all autotrophs or just a subset.
Response: Thank you for these comments. We believe these points are addressed in our responses to earlier, related comments above. To briefly reiterate: we clarified that all plant data in our study refer specifically to leaf tissue, and that autotroph and heterotroph samples were compiled independently. We also now more clearly distinguish between "plants" and "autotrophs" in the text to avoid any confusion. Furthermore, we have expanded the discussion to acknowledge both the similarities and discrepancies between our results and previous studies, including potential explanations for the absence of a latitudinal trend in plant nitrogen content.

L. 253: The discrepancy in environmental controls on plant N and P compared to previous studies is puzzling. Observed global patterns in plant nutrients are well-established and robust, which casts doubt on the conclusions of this study. One potential source of bias is the misestimation of N and P availability. Additionally, it is unclear which plant functional types and land-use categories were sampled. Factors such as fertilization or land use can override latitudinal patterns, further complicating interpretations. For these reasons, I remain unconvinced by the explanations provided by the authors.
Response: We appreciate the reviewer's concern and the opportunity to clarify this point. It is correct that terrestrial autotrophs in our dataset are overwhelmingly composed of vascular plants—specifically, 76% of all terrestrial autotroph records are from the class Magnoliopsida (see Methods and L425–429). To avoid confusion, we have revised the text where necessary to distinguish between "autotrophs" and "plants" and now specify when patterns are primarily driven by plant data.
We have made the following clarifications in the manuscript:

L231-233: "Notably, these patterns were largely driven by terrestrial autotrophs, predominantly composed of plants (see Methods section), which exhibited, on average, a 173% increase in P content (back- transformed scale) from 0° to 80° absolute latitude (Fig. 2C and Supplementary Table 1)."

L234-236: "Consistent with our predictions, terrestrial organisms exhibited a decline in N:P with increasing latitude (Fig. 3A and Supplementary Table 1), primarily driven by an increase in the P content of autotrophs (Fig. 2A, Fig. 3B and Supplementary Table 1)."
L251–254: "We observed a positive relationship between organismal N content and N deposition (Fig. 4A and Supplementary Table 1), driven predominantly by autotrophs (Fig. 4B, Supplementary Table 1) and freshwater organisms (Fig. 4C and Supplementary Table 1)."

With regard to the discrepancy between our findings and earlier studies on plant N content, we acknowledge that fertilization, land use, and sampling heterogeneity can indeed modulate or obscure latitudinal patterns. While these factors were not consistently

reported across the primary sources in StoichLife, we agree they are important sources of variation and have now addressed this explicitly in the discussion (L280):
"The consistent latitudinal patterns in plant P content and N:P ratio in our study and prior work11,12,51,63 point toward underlying global-scale mechanisms shaping autotroph stoichiometry. In contrast, the lack of consistent effects of latitudinal responses of plant N content suggests that local or context-specific factors, such as soil or water fertilization or land-use variation, may blur broader biogeographical patterns."

We also emphasize that while we did not observe a strong latitudinal trend in plant N content, our findings **do** align with earlier studies in terms of plant P content and N:P ratios (e.g., Reich & Oleksyn, 2004; Borer et al., 2013). The differences in plant N may reflect real variation across regions and land uses, or differences in analytical methods, as also suggested by the low R^2 values in earlier studies (e.g., $R^2 = 0.04$ in Reich & Oleksyn, 2004). More detailed information on taxa, sampling coverage, and data sources is provided in the StoichLife data paper and dataset, which are now more prominently referenced.

Figure 1: provide units for the parameters analyzed. Specify temperature.
Response:
Thank you for these observations. We have now added units to all relevant axes in Figures 1, 3, and 4, as well as in the corresponding Extended Data Figures. Temperature is now clearly specified in Figure 1, and N deposition is labeled with appropriate units throughout.

Figure 3/4: are the molar or mass ratios
Response: We have also clarified in the figures that N:P ratios are presented as molar.

Figure 4; extended Figure 3, 4: provide units of N availability on axis.
Response: N deposition is labeled with appropriate units throughout.

Extended Data Figure 3. Pearson correlation requires normally distributed data, but this does not seem to be the case.
Response: Thank you for catching this. We agree that Pearson's correlation assumes normally distributed data. To address this, we have reanalyzed the relationships using the Spearman correlation test, which is non-parametric and does not require normality. The results remain consistent with our original conclusions. We have updated both the text and figure captions to reflect this change.

======END OF REVISONS======

We thanks the reviewers for the constructive feedback on our manuscript. We have re vised the text carefully in response to both sets of comments and the editorial requests . Below, we provide a point-by-point response. All line numbers refer to the revised m anuscript.

<u>Reviewer #1</u>

General comment: The authors' responses demonstrate a good effort to address the reviewers' concerns and improve the manuscript. However, some responses are more convincing and thorough than others. The authors need strengthening those that are less direct or that potentially bypass the core issue raised by the referees.

Response: We are grateful for this assessment and have worked to strengthen our responses where flagged, particularly regarding (1) title specificity, (2) the broader stoichiometric framework, and (3) alternative explanations for protein synthesis limitation. In each case, we aimed to be more self-reflective and to clarify both the scope and limitations of our study.

1. Title specificity

Comment: It is true that similar titles exist, but the referee is highlighting a lack of specificity. Instead of only citing similar titles, you should briefly explain why a more specific title is undesirable in your context.

Response: We appreciate the reviewer's concern about the phrasing of the title. We recognize that our original response did not sufficiently explain why a more narrowly specific title is undesirable. Our intention is not to imply a direct mechanistic link between cellular metabolism and N:P ratios, but rather to emphasize that nitrogen deposition consistently emerges as the strongest environmental predictor of global variation in organismal N and P content across taxa, realms, and latitudes. A title centered narrowly on RNA–protein synthesis trade-offs or phosphorus limitation would not capture the full scope of our dataset and analyses, which for the first time integrates plants and animals across both freshwater and terrestrial systems. We therefore prefer a concise title that conveys the central macroecological finding while leaving room in the text to address the underlying physiological and biogeochemical mechanisms. To avoid implying direct causality, we have revised the title to "***Nitrogen deposition reveals global patterns in plant and animal stoichiometry***" . This formulation maintains clarity while also addressing Reviewer #2's concern about our use of "drives". Compared to previous studies, our title is also more specific in two respects: (i) it explicitly includes both plants and animals, and (ii) it extends beyond simple latitudinal gradients to evaluate global environmental predictors. We also believe this framing echoes long-standing goals in ecological stoichiometry to identify and understand patterns in organismal elemental content. Taken together, the revised title more accurately reflects the scope and contribution of our work.

2. Broader stoichiometric perspective

Comment: Could you discuss how including other elements would alter some of your findings? Could you provide more info on how you may incorporate the additional elements without a large data set?

Response: We thank the reviewer for this very insightful comment and apologize for initially misunderstanding its intent. We agree that situating N and P within a broader stoichiometric framework strengthens the manuscript.

Because elemental content is expressed as percentages of dry mass, values are bounded to 100%, and all unmeasured elements are implicitly represented as "other." This means that variation in %N or %P can partly reflect changes in other elements, such as C, K, or Ca. Explicitly incorporating these elements would help refine mechanistic interpretations by clarifying positive covariances or trade-offs (e.g., structural vs. metabolic investment), nutrient storage strategies, and physiological regulation. Such multidimensional approaches have been developed in both theoretical and empirical contexts, for example, the stoichiometric niche framework and the biogeochemical niche concept. These frameworks show that C content can illuminate structural versus metabolic allocation, while K and Ca can reveal additional constraints on osmotic balance, homeostasis, or skeletal investment (in vertebrates). Although we expect the dominant influence of N deposition on %N and %P to remain robust, multi-element data could uncover new mechanisms underlying stoichiometric variation.

We have further developed this point as a seventh limitation in the Discussion (Lines 386-396):

"Seventh, our analyses were limited to N and P, reflecting the availability of data. Because elemental content is expressed as percentages of dry mass, values are bounded to 100%, and unmeasured elements are implicitly represented as "other." This means that variation in %N or %P may partially reflect reallocations relative to other elements (e.g., carbon, potassium, calcium). While this does not undermine the robustness of our findings, it constrains mechanistic interpretation. Broader multi-element data would allow evaluation of covariation and trade-offs among elements, providing a more complete view of organismal stoichiometry across realms. Although multi-element datasets remain limited, smaller-scale studies already highlight the value of multi-element approaches. Incorporating such data in regional or taxon-specific contexts represents a feasible step toward testing multidimensional stoichiometric predictions and informing the gradual expansion of global databases."

We have also revised the Conclusion to emphasize these points (Lines 402-412):

"In conclusion…Expanding stoichiometric analyses to include elements beyond N and P will be critical for refining interpretations of how environmental drivers structure global patterns. In the near term, regional syntheses and taxon-specific studies can help bridge the gap until broader global coverage is achieved. Future progress will benefit from multidimensional stochiometric frameworks (e.g., stoichiometric[72,73] and biogeochemical niches[74,75]), which conceptualize elemental content in a multivariate trait space and enable assessment of covariation, trade-offs, and environmental sensitivity across scales. Such integrative approaches, coupled with expanded global datasets linking organismal and resource stoichiometry, will improve predictions of how

human-driven changes in nutrient inputs and climate affect the elemental content of life and, ultimately, multiple dimensions of biodiversity."

At present, comprehensive datasets for other elements are lacking, which constrains direct integration at the scale of our study. However, smaller-scale datasets, targeted experiments, and theory-driven simulations already demonstrate the value of multi-element approaches. Incorporating such data through regional syntheses or focused case studies would allow us to test predictions from multidimensional frameworks and inform the gradual expansion of global datasets.

We hope these additions better address the reviewer's concerns.

3. Protein synthesis limitation
Comment: Consider adding a statement of alternative hypothesis.

Response: We thank the reviewer for this constructive suggestion. We acknowledge that our earlier framing was too narrow and have now expanded the discussion to explicitly recognize alternative nutrient-related mechanisms that could explain variation in % N, % P, and N: P. The revised text (Lines 247-255) reads:

"The observed decline in N:P has often been attributed to the greater environmental sensitivity of P-rich ribosomes compared to N-rich proteins[54,55], consistent with the growth rate hypothesis[33]. While this metabolic explanation is plausible, alternative mechanisms may also contribute. For example, nutrient supply constraints, such as low soil P availability in highly weathered tropical soils and strong binding of P to iron (Fe) and aluminum (Al) oxides[56], could reduce autotroph P content at low latitudes. In addition, differences in nutrient storage capacity, lineage-specific allocation strategies, or species turnover along environmental gradients could also alter %N, %P, and N:P ratios independently of protein/RNA synthesis demands."

<u>Reviewer #2</u>

Comment: In their revisions, the authors have successfully addressed the comments. The manuscript provides an important, novel and timely assessment of global patterns of plant and animal stoichiometry, making use of a large unique data base. I have a few minor comments that might help to improve the manuscript.

Response: We thank the reviewer for this positive assessment and constructive suggestions.

More self-critical reflection/Quantify uncertainty: You address the referee's concerns well but try to also demonstrate critical self-reflection on your own methodology. Quantify limitations in the Data Set: Can you give a rough percentage estimate of missing data or the range of uncertainty for key variables? This would add to the transparency of your analysis.
Response: All samples included in this study contained complete measurements of %N and %P, since both values were required to calculate N:P. Consequently, the dataset

used in our analyses had no missing values for these variables. We recognize, however, that uncertainty arises from two other sources: (i) methodological differences among the original studies contributing to the StoichLife database (González et al. 2025), which all followed established protocols in plant and animal stoichiometry research, and (ii) the environmental proxies used (e.g., modeled nitrogen deposition, labile P), which provide globally consistent coverage but may not fully capture local nutrient availability.

To address these uncertainties, we used permutation-based tests and conducted sensitivity analyses that removed random subsets of locations and species. Both approaches confirmed that our results were robust to methodological variation, sampling bias, and proxy-related uncertainty.

We clarified these points in the Methods (Lines 443-445):

"Only records with both %N and %P were retained, as these values were required to calculate N:P. As a result, the dataset contained no missing values for these variables. Variation across studies reflects methodological differences in elemental analysis, but all unpublished data followed established protocols widely used in plant and animal stoichiometry research."

And in the Statistical Analyses section, we have added more details on the models (Lines 503-505):

"In total, we built six models, each with varying numbers of observations (models with latitude / environmental drivers): logit-transformed %N (N = 22,467 / N = 19,040), %P (N = 11,200 / N = 8,479), and log-transformed N:P ratios (N = 9,253 / N = 6,777).

And also added a statement about how uncertainties were handled (Lines 541-544).
... Importantly, these permutation and sensitivity tests also serve to evaluate robustness to potential measurement variation and uncertainty in environmental proxies, confirming that observed relationships were not artefacts of data structure."

1. Nitrogen deposition as proxy and highlighting a key finding
With respect to nitrogen deposition, I would recommend presenting its use as a proxy for nitrogen availability and limitation early on, rather than hiding it. You may present it already in the methods section of the 'Nutrient-Dependent Physiology' part of the introduction (lines 172–182). I think one of the key findings requires more prominence and critical reflection.

Response: We thank the reviewer for this helpful suggestion. We agree it is important to state explicitly and early that nitrogen deposition was used as a proxy for environmental nitrogen availability. To address this, we have revised the Introduction (Lines 212–214):

"In this study, we used atmospheric N deposition as a proxy for environmental nitrogen availability and limitation, acknowledging both its strengths as a globally consistent metric and its limitations relative to local soil and water nutrient supply."

We also emphasize this in the Methods (Lines 468-469), and provided a more explicit critical reflection in the Discussion (Lines 317-323).

"Finally, we emphasize that N deposition was used here as a proxy for environmental N availability and limitation. This proxy provides globally consistent coverage and captures large-scale enrichment patterns, but it does not necessarily reflect local variation driven by soil chemistry, microbial processes, or land-use history. The fact that N deposition nonetheless emerged as the strongest environmental predictor highlights both its utility as a broad-scale indicator and the need for complementary, finer-scale measures of nutrient availability in future work."

2. Minor corrections

- **Line 146:** punctuation **corrected**
- **Line 244:** rephrased "near the poles" to "at higher latitudes."
- **Line 365:** added detail on Fe/Al oxides binding P in tropical soils.
- **Line 374:** added "soil properties" to the list of modulating factors.

Comment: Overall, a more nuanced use of 'driven': I recommend using it only if there is a 'driving' mechanism, and not if other mechanisms 'cause' a certain pattern.
For example, at line 228, '...our results suggest that variation in N:P stoichiometry with latitude is primarily driven by changes in P content.'
In this case, it would be more appropriate to use 'is related to' or 'is caused by'.
This applies for more sentences.

Response: We recognize that our original use of the term "driven" may have implied a stronger causal interpretation than was intended. To address this, we now use "related to" or "associated with" where no direct mechanism is implied. This revision also applies to the title, which has been updated from *"**Nitrogen deposition drives global patterns in plant and animal stoichiometry**"* to*"**Nitrogen deposition reveals global patterns in plant and animal stoichiometry**"*

Summary - We believe these revisions thoroughly address the reviewers' and editor's concerns and improve the clarity, precision, and scope of the manuscript. We thank the reviewers for their constructive feedback, which helped us refine framing, acknowledge key limitations, and strengthen the overall contribution. We hope the revised version will now be acceptable for publication in *Nature Communications*.

--------------------END OF COMMENTS----------------