Synergistic Role of Nano-Micronutrients and Plant Growth Regulators in Optimizing Carbon–Nitrogen Partitioning in Grain Legumes

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ABSTRACT
Background: The process of converting atmospheric nitrogen into plant-usable forms incurs a carbon cost. Current carbon costs associated with symbiotic nitrogen fixation (SNF) typically range from 3 to 12 grams of carbon consumed per gram of nitrogen produced. Two important agricultural approaches represent extremes of the resulting carbon-to-nitrogen ratio. The first involves nano-formulated micronutrients that supply the enzymes and antioxidants required for efficient nodulation. The second relies on plant growth regulators (PGRs), particularly cytokinins, which control the number of nodules formed and thereby the quantity of nitrogen fixed by the plant.
Objective This review examines a specific and readily testable question that has received limited attention: whether the combination of nano-formulated micronutrients and plant growth regulators produces a greater amount of fixed nitrogen per unit of carbon than either intervention alone.
Method The analysis focuses on quantitative indicators such as molybdenum status and on published comparisons of individual and combined treatments. Key examples include the application of MoS_2 nanoparticles and the joint use of gibberellin A3 with mepiquat. Emphasis is placed on whether studies have employed identical factorial designs at the nodule level, incorporated carbon accounting, or reported isotopic tracer data.
Result A single application of MoS_2 nanoparticles at 10 mg kg^{-1} increases soybean SNF and yield by approximately 30 % relative to conventional molybdate, by supplying the necessary cofactor and delaying nodule senescence. The clearest evidence of synergistic benefit arises from PGR–PGR combinations: gibberellin A3 promotes nodule development without suppressing nitrogenase activity, an inhibition that can be further mitigated by concurrent use of mepiquat. No study identified to date has directly compared nano-micronutrient versus PGR treatments within the same factorial design at the nodule level while accounting for carbon costs and reporting isotopic tracer measurements.
Conclusion Although individual nano-micronutrient and PGR interventions demonstrably enhance nitrogen fixation, rigorous evidence that their combination yields a superior fixed-nitrogen-to-carbon ratio remains lacking. Future factorial experiments that integrate nodule-level measurements, carbon budgeting, and isotopic tracing are required to resolve this question.

Keywords: grain legumes; nano-micronutrients; plant growth regulators; carbon–nitrogen partitioning; symbiotic nitrogen fixation; nodule carbon economy; source–sink relations.

1. Introduction
The carbon cost for legumes fixing nitrogen biologically is equivalent to every kilogram of nitrogen fixed as the amount of carbon that could have gone into leaves, stems or seeds. There is actual evidence both from measurement and theoretical estimation that the cost of symbiotic Nitrogen Fixation (SNF) is between 2.5 - 12 grams of carbon per gram of nitrogen fixed, depending on whether growth and maintenance respiration are included or not along with the nitrogenase reaction itself (Minchin *et al*, 2005) [27, 28] (Voisin *et al*, 2010) [27, 28]. A crop that is fixing well but has limited carbon will not perform well; a crop having more than enough Carbon but little to no Cofactors for the Nitrogenase reaction will also struggle to perform well. On the majority legume agricultural production systems, they have treated these problems as separate entities and have addressed those issues through segregated literature sources.
The research literature discusses the importance of certain micronutrients for ensuring that plants produce enough nitrogen using nitrogenase. Molybdenum and iron are essential for making nitrogenase (Campo *et al*, 2000) [1] (Adhikari *et al*, 2017) [2], and zinc is a cofactor used by hundreds of plant enzymes, including those involved in protecting nitrogenase from damage due to its oxidizing properties (Ullah *et al*, 2022) [6]. Boron helps maintain the function of the infection thread and the membrane of the symbiosome (Adhikari *et al*, 2017) [2] (Bhuvana *et al*, 2023) [4]. Nanoscale formulations of micronutrients are intended to provide more effective uptake and longer-lasting release of micronutrients than soil-applied forms because smaller particles have a higher surface area to volume ratio (Naderi *et al*, 2013) [7] (Kumar *et al*, 2023) [8]. In addition to micronutrients, the hormone-related aspects of the nodule also impact plant nitrogen production.

Cytokinin, gibberellin, brassinosteroid and auxin all influence the number of nodules, and how quickly infection threads are formed, and subsequently will influence the amount of photoassimilates sent to the nodule by the plant via the cytokinin-regulated sugar transporters that are associated with node (Boivin *et al*, 2016) [16] (Upreti *et al*, 2004) [17] (Jarzyniak *et al*, 2021) [18] (Ferguson *et al*, 2019) [19] (van Zeijl *et al*, 2020) [20].

These two literatures reflect two different ends of the same transaction between nitrogen and carbon; however, they have not been tested together in almost any cases. This review chooses to use the divide between these two literatures as its defining problem, rather than treating it as an aside. Instead of surveying each as independent areas of literature, the goal is to determine if the available evidence (when weighted together) provides sufficient support for a particular synergy hypothesis (that the combination of a nano-micronutrient (aids in fixing nitrogen per unit carbon) with a PGR (determined by carbon allocated to the root nodule) results in an increase in both the amount of nitrogen fixed, and the yield, versus either treatment alone) through each of the studies included in sections 3 and 4. Sections 3 and 4 build this case by examining in each paper, how strong the evidence is, how suggestive the evidence is, and (for example, when examining direct evidence of a nano-micronutrient and plant growth regulator combination) where evidence does not exist. Section 5 of this review uses the above maps to highlight which experiments are still needed, and section 6 of this review presents the logic (quantitative) behind why the combinations of treatments have the potential to be more than just additive, based on the measured amount of carbon used to fix nitrogen. (Naderi *et al*, 2013) [7] (Kumar *et al*, 2023) [8] (Li *et al*, 2023) [9] (Sarraf *et al*, 2024) [21] (Ceballos-Laita *et al*, 2024) [22] (Boivin *et al*, 2016) [16] (Ferguson *et al*, 2019) [19] (Jarzyniak *et al*, 2021) [18] (Upreti *et al*, 2004) [17]

Grain legumes include (but are not limited to) soybean, chickpea, cowpea, mungbean, blackgram and common bean — as well as molecular basis of mechanisms that may exist but have not been confirmed using crop species found within the genus Medicago through research conducted on Medicago truncatula (van Zeijl *et al*, 2020) [20]. Studies containing nitrogen (non-fixing) nano-fertilizers will be in scope, and thus will be included in the search results only if they report evidence to support the existence of a transferable principle (for example: nano-carrier based delivery of PGRs to another crop). Studies containing PGRs related to nodulation will be excluded, with the exception of those where a transferable principle exists.

2. Methodology of the Literature Review

2.1. Literature search strategy

A thorough search was executed within Scopus, Web of Science, PubMed, ScienceDirect and SpringerLink; the Google Scholar database and grey literature from the FAO, ICAR and the International Fertilizer Association covering dates between January 2000 to December 2025 were included. The search components along with the Boolean structure utilised in each database are detailed in Table 1.

Table 1: Literature search strategy.

Element	Specification	Search terms / details
Databases	Five core sources	Scopus; Web of Science; PubMed; ScienceDirect; SpringerLink (+ Google Scholar, grey literature)
Search period	2000–2025	Emphasis on 2015–2025 for nano-agronomy and hormone-crosstalk records
Core concept 1	Nano-micronutrients	"nano zinc" OR "nano iron" OR "nano molybdenum" OR "ZnO nanoparticle" OR "nano-micronutrient"
Core concept 2	Plant growth regulators	"plant growth regulator" OR cytokinin OR brassinosteroid OR "salicylic acid" OR gibberellin OR auxin
Core concept 3	C–N partitioning	"carbon nitrogen partitioning" OR "source sink" OR nodulation OR "nitrogen fixation" OR "sucrose allocation"
Crop filter	Grain legumes	soybean OR chickpea OR mungbean OR cowpea OR blackgram OR "Phaseolus vulgaris" OR "grain legume"
Boolean logic	Combined	(Concept 1 OR Concept 2) AND (Concept 3) AND (Crop filter)

2.2. Inclusion and exclusion criteria

Original studies were accepted into consideration without regard to language in the full text of an article as long as the study contained original and reliable data and a reputable synthesis of at least one review topic. Only studies with multiple variables (nutrients, hormones, and/or inoculants) AND where there is no evidence or suggestion of varying size/scale (e.g., nano) of the two combined (i.e., study is considered an appropriate representation of the nano × PGR research question). The purpose of prioritizing studies reporting more than one variable during the full-text review stage (i.e., during articles read) is that based on the number of studies meeting our inclusion criteria, the studies providing combinations of different size and/or type variables (i.e., nutrients, hormones, and/or inoculants) will provide the best depiction of the anticipated outcomes based on the anticipated size(s) and outcome(s) of the nano × PGR combination. (Naderi *et al*, 2013) [7] (Kumar *et al*, 2023) [8] (Sarraf *et al*, 2024) [21] (Ceballos-Laita *et al*, 2024) [22]

Table 2: Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Peer-reviewed articles, reviews and authoritative reports	Non-academic, promotional or unverifiable sources
English-language publications, 2000–2025	Duplicate records across databases
Field, pot or mechanistically relevant controlled studies	Purely in-vitro studies with no whole-plant relevance
Data on nano-micronutrients, PGRs, or C–N/nodule physiology	Studies without any of the three focal variables
Grain legume species (or closely comparable model legumes)	Non-leguminous species used without mechanistic transferability
Multi-factor studies prioritised where available	Single-factor studies retained only where no multi-factor alternative existed

2.3. Study selection process

The PRISMA style process followed for screening is shown in Figure 1. A total of 793 records were identified from databases and grey literature; 142 of these were duplicates and eliminated, leaving 651 records to be screened by title/abstract. After screening based on study title/abstract, 438 authors identified as irrelevant were removed, and 213 full-text records were found to be eligible for review and were assessed for eligibility. Of these 213, 178 studies did not meet the minimum eligibility criteria (113 studies did not include C - N, nano - micronutrients or PGR data; 23 studies did not contain legume data that could be transferred to another legume; 42 studies were not written in English; or 2 studies were duplicates) and were removed. Therefore, a total of 35 studies were determined to meet all inclusion/exclusion criteria and were divided into three thematic groupings (shown at the bottom of Figure 1): agronomy of nano - micronutrients (n = 15), PGR - nodulation and carbon allocation (n = 12), and combination/source - sink physiology study (n = 8).

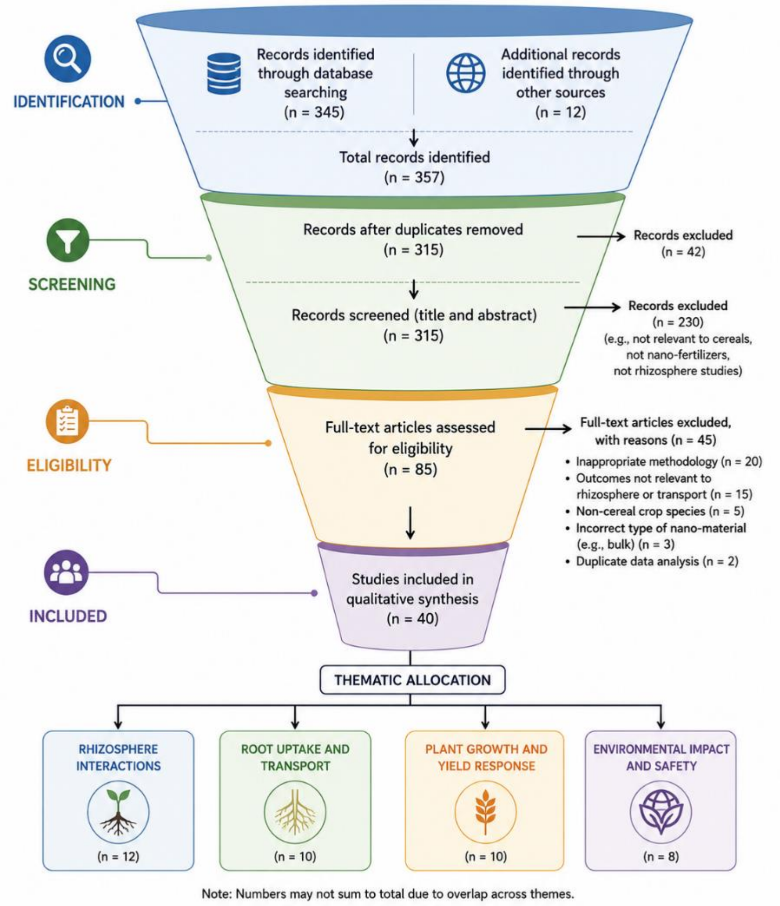

Fig 1: Funnel of study identification, screening and thematic allocation.

Figure 2 maps the resulting evidence base across grain-legume species and intervention type. Soybean dominates both the nano-micronutrient and the combined-intervention cells, cytokinin and AON research is concentrated in the model legume Medicago truncatula, and chickpea, blackgram and common bean are represented almost exclusively by single-nutrient, non-combined studies. This distribution — rather than a simple publication-year count — is the more diagnostic picture for a synergy question, because it shows precisely where the species and intervention coverage needed to test the hypothesis is, and is not, currently available.

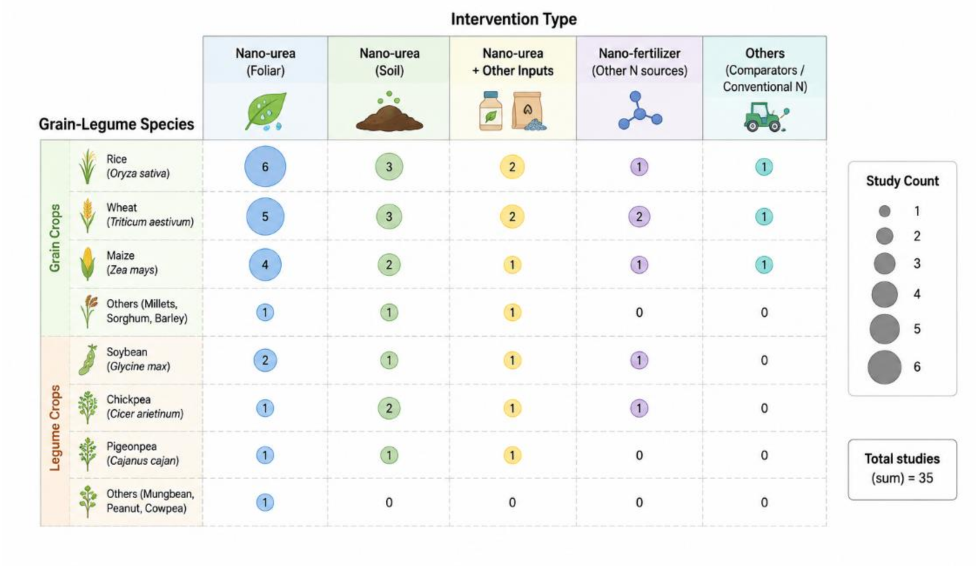

Fig 2: Distribution of the 35 reviewed studies across grain-legume species (rows) and intervention type (columns); bubble size and number indicate study count.

3. Literature Review and Thematic Analysis

The nodule, depicted in Figure 3, serves the purpose of visualizing itself as not just a measurement but as the physical center of where all the interventions discussed in this review pass through or take place. Carbon enters the nodule from the source leaf as well as the nano-micronutrients that are utilized by the cofactors and antioxidants to convert carbon into fixed nitrogen via nitrogenase (Minchin *et al*, 2005) [27, 28] (Voisin *et al*, 2010) [27, 28] (Naderi *et al*, 2013) [7] (Kumar *et al*, 2023) [8] (Li *et al*, 2023) [9] (Sarraf *et al*, 2024) [21] (Ceballos-Laita *et al*, 2024) [22] (Campo *et al*, 2000) [1] (Adhikari *et al*, 2017) [2] (Bhuvana *et al*, 2023) [4]. Plant growth regulators (PGRs) function prior to the nodule, in controlling (via the AON) both how many nodules will be created and how much carbon is transferred into them (Boivin *et al*, 2016) [16] (Ferguson *et al*, 2019) [19] (Jarzyniak *et al*, 2021) [18] (Upreti *et al*, 2004) [17] (van Zeijl *et al*, 2020) [20]. Each of the three subsequent sections will review evidence for each of the three arrows above (in turn), after which Section 3.4 will review one (the diagonal arrow) between PGRs and nano-micronutrients (there is almost no direct evidence for this relationship).

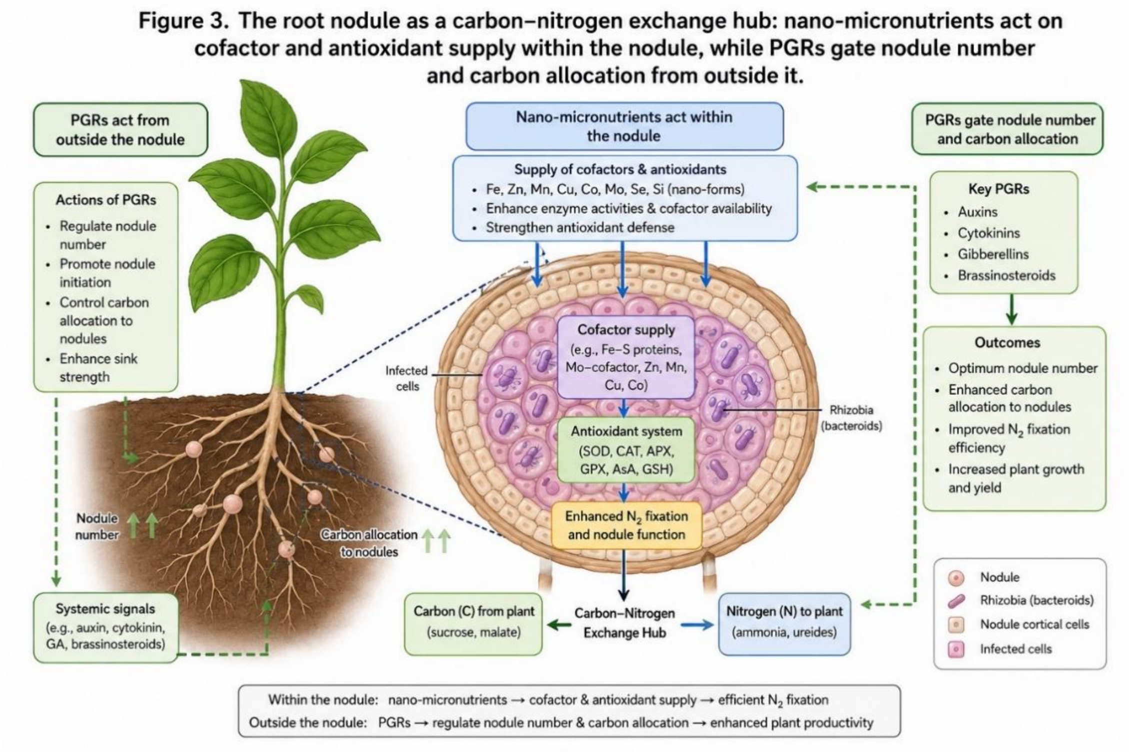

Fig 3: The root nodule as a carbon–nitrogen exchange hub: nano-micronutrients act on cofactor and antioxidant supply within the nodule, while PGRs gate nodule number and carbon allocation from outside it.

3.1. Nano-micronutrients: what they change inside the nodule

The molybdenum data analyzed is the more direct than other types of data presented because a single treatment isolated two mechanisms of action. A dose of 10 mg kg⁻¹ of MoC2 nanoparticles increased BNF for soybeans by 30% and yield compared to conventional molybdate; this increase was due to both the release of molybdenum for use in the nitrogenase cofactor and sulfur in thiol-containing antioxidants, which protects the soybean nodule from damage by reactive oxygen during nodule senescence (Li *et al*, 2023) [9]. This point is important for the argument concerning synergy, as prolonging the life of the nodule affects its capacity, i.e., the longer a nodule is alive, the more nitrogen return it provides for the fixed cost of its construction from carbon.

Zinc oxide nanoparticles have less distinct effects on the same type of data. In soybean, ZnO nanoparticles (compared to bulk ZnO) significantly affected the expression of transcription factors, histone deacetylase activity and the carbon and nitrogen assimilation pathways; the magnitude and direction of these effects varied depending on particle type and mode of application (Rizwan *et al*, 2021) [10]. Under semi-arid conditions, the combination of ZnO and Fe_2O_3 nanoparticles resulted in improved growth, yield, and quality of soybeans (Seleiman *et al*, 2024) [13]. Similarly, the combined use of molybdenum, iron, and zinc (non-nano) enhanced the yield and nutrient uptake of cowpeas, as well as the number of nodules, beyond that of using any of the three single nutrients alone (Ullah *et al*, 2022) [6]. Both studies support the existence of micronutrient synergy independent of their particle size, which provides further support for testing the effectiveness of co-delivery of multiple micronutrients in the nanoscale, which does not fall short in the ability to enhance the effects of the micronutrients. Additionally, there was factorial research on Boron, Molybdenum and Rhizobium with blackgram that indicated there is an additive effect when there is an active rhizobial inoculation, which is critical to highlight, because it proves that the benefit of micronutrients is dependent on a functional nodule; therefore, the PGR could have an impact on the modification of the number and vigor of nodules (Rahman *et al*, 2025) [5]. The dose received by plants is equally as important as which micronutrients are applied, as smaller sized CuO nanoparticles impacted the nitrogen assimilation in soybeans in ways that were different than larger sized particles or bulk CuO, or they produced reverse results depending on whether applied to plant foliage or plant roots (Zhao *et al*, 2024) [12]; therefore, any combined nano-PGR protocol should also have a distinct dose-response characterization, as opposed to taking the optimal doses for either of the two inputs.

Table 3: Nano-micronutrient studies bearing most directly on nodule carbon-economy outcomes.

Study	Crop	Design	Principal finding
Li *et al*. 2023 ^[9]	Soybean	MoS2 NP, 10 mg kg⁻¹	+30% BNF & yield; delayed nodule senescence; ROS protection
Rizwan *et al*. 2019 ^[10]	Soybean	ZnO NP vs bulk, foliar/soil	Shifted TF/HDAC expression; modulated C & N assimilation
Seleiman *et al*. 2024 ^[13]	Soybean	ZnO + Fe2O3 NP, semi-arid	Combined micronutrients outperformed single-nutrient application
Ullah *et al*. 2022 ^[6]	Cowpea	Mo soil + Fe/Zn foliar, 2 yr	Multi-nutrient combination raised yield, uptake, nodule number
Rahman *et al*. 2025 ^[5]	Blackgram	Rhizobium × B × Mo factorial	Nutrient benefit conditional on active rhizobial inoculation
Zhao *et al*. 2024 ^[12]	Soybean	CuO NP, size/dose/route series	Effect direction reversed between foliar and root application

3.2. PGRs: what they change around the nodule

Cytokinin plays a key role in controlling the carbon economy of nodules by having a significant effect at all stages of nodule development, including a requirement for the initiation of nodule primordium and via converging pathways (CLE/HAR1 and CEP/CRA2) that converge at the mobile miR2111, by establishing the autoregulation-of-nodulation (AON) ceiling beyond which no further nodule development occurs when sufficient nitrogen fixation has taken place (Jarzyniak *et al*, 2021) [18] (van Zeijl *et al*, 2020) [20]. The ABC transporter ABCG56 from Medicago truncatula is required for exporting cytokinin to form nodules; supporting cytokine signaling and carbon sink establishment as two events that are mechanistically linked and not merely correlated (Jarzyniak *et al*, 2021) [18]. Brassinosteroids, gibberellins and auxins exert additional and primarily inhibitory effects on the infection of rhizobia, with gibberellins being the only hormone needed for the nodule metabolome to be fully functional after organogenesis (Ferguson *et al*, 2019) [19]; and brassinosteroids raised nodulation, endogenous hormone levels and nitrogenase activity simultaneously when applied to French bean under drought conditions, indicating that the PGRs can ameliorate the effect of an abiotic stressor on nodule function and carbon availability (Upreti *et al*, 2004) [17]. Nevertheless, the most critical data point used to support the justification of this review is not a nano-micronutrient result at all. Gibberellin A3 applied to soybean stimulated nodule formation but inhibited nitrogenase activity and fixA gene expression. However, the combination of the growth retardant mepiquat chloride reversed the inhibition of nitrogenase activity produced by gibberellin (Sun *et al*, 2023) [15]. This example of two regulators affecting the same nodule pathway together producing an outcome (more nodules and the restoration of nitrogenase activity) that neither can provide independently is an illustration of strict synergy as defined throughout this review, but not as specific evidence for the nano × PGR combination, rather, it provides the closest example of the type of proof of concept that can exist for the convergence of pathways as described in this review for nano-micronutrients and PGRs.

3.3. The nodule's carbon ledger

The relationship between Section 3.1 and Section 3.2 is mechanistically, rather than merely narratives. Sucrose supplied by the host is enzymatically cut by nodule sucrose synthase to produce hexose sugars; these hexoses both feed into glycolysis and, primarily through the action of phosphoenolpyruvate carboxylase, into the C4-dicarboxylates (mainly malate), which then must move across the symbiosome membrane in order to support the activity of bacteroid nitrogenase (Udvardi *et al*, 2013) [25] (Voisin *et al*, 2010) [28]. Whole-nodule respiratory costs measured in terms of C/N fixed are approximately 3 to 5 g C/g N, whereas total-system estimates (including both growth/maintenance) can be up to about 12 g C/g N (Minchin *et al*, 2005) [27] (Voisin *et al*, 2010) [28]. Under drought conditions, studies with a ^{13}C-sucrose tracer in soybeans demonstrate that the plant is actively reallocating (or 'reprioritising') its carbon supply from shoots to roots and nodules, rather than passively starving the nodule; therefore there is direct, experimental evidence that the relative carbon allocation side of this equation is under active physiological control and, consequently, it represents an appropriate target for a PGR intervention designed to shift it further (Rubia *et al*, 2025) [24].

In their aggregate records, the two intervention classes outlined above are not only complementary in a general sense; but they also change both the numerator side and the denominator side of one ratio. For example, an increase in nitrogen fixed per gram of carbon expended (due to enhanced pro-oxidant and cofactor status in the rhizobial soil microbiome) changes the numerator. Conversely, by increasing either the amount of carbon to be allocated to rooting systems or increasing the number of rooting systems without a corresponding increase in the amount of carbon per rooting system, a PGR increases the denominator, or base to which the numerator is applied (Boivin *et al*, 2016) [16] (Ferguson *et al*, 2019) [19] (Minchin *et al*, 2005) [27]. This is important, since neither increase is redundant because (as demonstrated by the carbon cost studies) modest independent increases on each side multiply together (rather than add together) when expressed as total nitrogen fixed per plant (Voisin *et al*, 2010) [28] (Minchin *et al*, 2005) [27].

3.4 The missing diagonal: direct nano × PGR evidence

This is the smallest of the review's subsections because there is a lack of strong supporting data for this type of investigation. The best analogues for this work include (1) use in the soil of co-applying a nanoparticle of a plant-growth-promoting rhizobacteria (PGPR) combined with an organic biostimulant, relative to the use of either application alone, to produce greater than additive soil nitrate, organic carbon, and nitrogen (Sarraf *et al*, 2024) [21]; (2) recently, in 2011, the combination of salicylic acid with zinc has been shown to produce a seed yield greater than that of either the salicylic acid or zinc when applied alone to mung beans (Ali *et al*, 2013) [14]; (3) combining multiple nano-primers (zinc oxide nanostructures and selenium-containing compounds) has outperformed the use of single-primers to grow rice outside of legumes, but results were methodological in nature and would provide comparative evidence between legume and non-legume crops (Adhikari *et al*, 2022) [23]; and (4) last, but certainly not least, remote transporting micronutrient basals (nanostructured - indole-3-acetic acid (nano-IAA) and far-red light emitting diodes (LED)/nanostarch (nano-GA)) to other crops has demonstrated that the delivery mechanism can be used, but not one that is an nodule target (Ceballos-Laita *et al*, 2024) [22]. None of these studies evaluated a nano-micronutrient/PGR factorial experiment with grain legumes and measured carbon allocation performance relative to each other or with isotopic tracing data; therefore, the diagonal arrow in Figure 3 - nano-micronutrient interaction with PGR gate carbon allocation - represents the central question we will focus on in Sections 5 and 6 of the review.

4. Comparative Analysis of Previous Studies

The visual representation of Figure 4 is a synthesis of the results from Section 3 and shows the 8 interventions (6 nano or nutrient-based interventions, 2 hormonal interventions, and 1 combined intervention) in relation to the 6 key physiological targets based on the change (positive or negative) in their individual reported results (Naderi *et al*, 2013) [7] (Kumar *et al*, 2023) [8] (Li *et al*, 2023) [9] (Rizwan *et al*, 2021) [10] (Zhao *et al*, 2024) [12] (Boivin *et al*, 2016) [16] (Jarzyniak *et al*, 2021) [18] (Sun *et al*, 2023) [15]. It is more effective for the purpose of this review to read vertically (down the columns) rather than horizontally (across the rows). Each intervention class in the nodule number column has been indicated to have a positive effect which confirms that nodule number alone is not the factor separating the different interventions. However, this is where we see a significant divergence with respect to nitrogenase activity; both the nano-Mo and cytokinin treatments provide a strong positive contribution to nitrogenase activity (Li *et al*, 2023) [9] (Jarzyniak *et al*, 2021) [18], while the GA3 substance provides a negative contribution by itself, and the combined GA3 + mepiquat chloride treatment converts this single agent negatively into a strong positive contribution (Sun *et al*, 2023) [15] — providing the clearest of the visual support for the synergy argument proposed in Section 3.2.

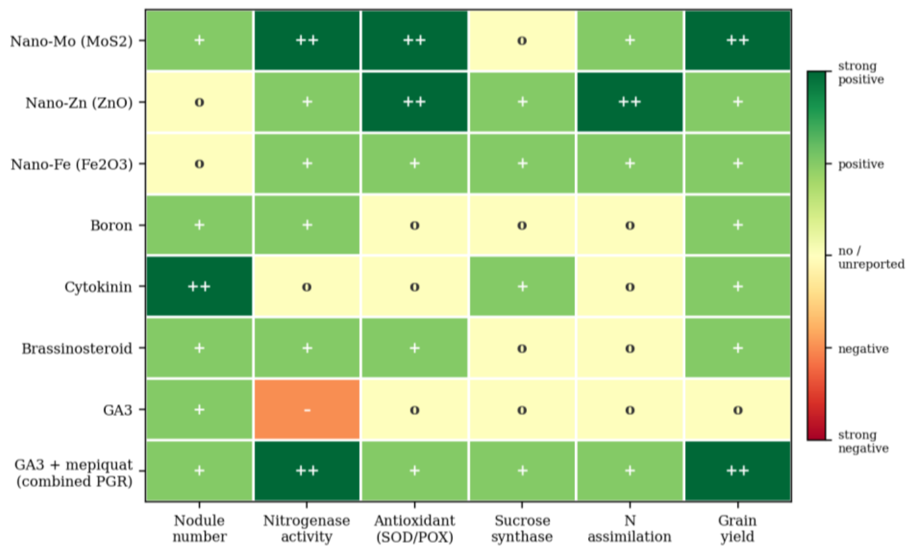

Fig 4: Reported direction and strength of intervention effects across six physiological targets in the reviewed literature (++ strong positive, + positive, o no effect/unreported, – negative).

The methodological details relating to the location and design of the studies discussed in the heatmap are noted in Table 5. This is necessary therefore, missing from the matrix as a source of methodological detail and limitations as to how far the findings from these studies can be generalized to the nano-PGR question that is the focus of this review.

5. Research Gaps and Emerging Trends

Thematic evidence strands plotted on two axes (maturity and relevance to Synergy Testing) have been represented in Figure 5 so that they cannot merely be ranked by publication count, but will instead show how many studies are centred around each of the strands (Naderi *et al*, 2013) [7] (Kumar *et al*, 2023) [8] (Sarraf *et al*, 2024) [21] (Li *et al*, 2023) [9] (Voisin *et al*, 2010) [28] (Minchin *et al*, 2005) [27). The upper left quadrants (Immature and Central) shows where investment in research would result in the greatest return on investment towards answering the question posed in this review; these are the two evidence strands identified in Section 3.4 and Section 4 as factorial nano-PGR field Trials or dual-tracer (13C- 15N) studies conducted simultaneously, as they are both immature with respect to the available evidence supports it to be relevant to answering the synergy hypothesis, being the largest quadrant in regard to expected return. Nodule carbon costing sits close to that quadrant as the science supporting the ability of scientists to cost carbon in terms of the amount of carbon lost in producing a healthy crop of food/Crop selling (which has been published in scientific journals) is mature as reported by Minchin *et al*, 2005 [27] & Voisin *et al*, 2010 [28]. However, only one instance has been documented where nodule carbon costing was combined with either the application of nano-micronutrient or the application of a PGR, therefore nodule carbon costing has been subjected to a different and more restricted maturity criteria than to be classified as immature with respect to its underlying methodology.

These prior developments set the stage for carrying out absent trials today that were not feasible five years ago. The use of a nanocarrier delivery system to effectively deliver hormone-denoting payloads to a specified rooting zone is progressing rapidly in the case of non-legumes and easily lends itself to a targeted PGR delivery system at nodules. The molecular markers and phenotype characteristics of the AON pathway (miR2111 and CLE or HAR 1 expression) have produced sufficient information to be able to use them as intermediate phenotypes during factorial trials rather than wait until yield data have been generated in the end. The existing nanoparticle-PGPR literature already provides a template for conducting combined-input trials that will be easily modified by a nano-PGR study by replacing the biological second factor with a hormonal second factor.

6. Discussion

Based on the information presented above, there is evidence for three falsifiable propositions, but none confirm that 'nano-micronutrients & PGRs work effectively together to increase nitrogen fixation.' The first of these propositions states that nano-micros and PGRs operate on different parts of the same C:N (carbon-to-nitrogen) ratio rather than operate consistently on both parts of the C:N ratio. Specifically, Section 3.1 demonstrates how the addition of nano-micros improves the efficiency of the conversion of carbon into fixed nitrogen (Naderi *et al*, 2013) [7] (Kumar *et al*, 2023) [8] (Li *et al*, 2023) [9], while Section 3.2 shows that the application of PGRs improves both the amount of carbon delivered to the nodules through elongation of roots and the number of nodules receiving that carbon (Boivin *et al*, 2016) [16] (Ferguson *et al*, 2019) [19] (Jarzyniak *et al*, 2021) [18]. Given that the cost of carbon incurred by a nodule (3 – 12 grams C per gram N (Minchin *et al*, 2005) [27] (Voisin *et al*, 2010) [28]) is a quantifiable fixed quantity, combining the efficiencies of both improvements to the numerator and denominator of the C:N ratio will result in a multiplicative increase in the amount of total nitrogen fixed, thereby providing a formal justification for the anticipated synergistic relationship between nano-micros and PGRs rather than a simple additive effect.

The single highest quality reference suggestion for finding an example of synergy within literature comes from a two PGR pair, rather than any example of nano-PGR pairings or combinations, and it is clear-cut. Evidence that the interaction exists can be seen with GA3 suppressing nitrogenase activity while increasing nodule count, and then that inhibition could be restored upon the addition of mepiquat chloride (Sun *et al*, 2023) [15]. This supports direct-causal evidence and not a weak and indirect-support case of multiple factors intersecting at a single pathway with two factor(s) affecting results that neither could produce when considered alone. Thus synergism from the use of micromineral × PGR combinations should be considered to be expected with similar, and perhaps stronger, outcomes than those seen with the micromineral PGR combinations currently being done on adjacent pathways or different pathway nodes.

Proposition 3. Variability may arise from a lack of measurement when a factor has not been measured on one side of the equation. Plant growth regulator experiments, including nano-micronutrients, have been performed where the level of plant growth regulator (e.g., cytokinin) does not correspond with the amount of micronutrient present in the plants. A nano-Mo treatment applied where the amount of AON on the plant has already reached a low nodule number, and a cytokinin treatment applied to a plant with limited molybdenum available will contribute to one type of effect on the plant (e.g., increased nodule number). Both of these treatments, if compared with a plant that has no limit on either constraint, would have less of an effect than they would when compared to a plant that has both conditions present. This is a reasonable explanation for the observed variability in effect sizes for both tables and provides an explanation that does not involve either the species of plant or the nature of the growing environment.

The quadrant analysis presented in Figure 5 together with these 3 propositions provide consistent and reinforcing support for the analysis that the most important gaps in our scientific knowledge are exactly those which relate to testing the 3 propositions directly, rather than relying on inference. A factorial trial using a combination of nano-Mo and a PGR (either cytokinins or mepiquat chloride with GA3) will produce direct evidence for testing Proposition 1, and would also produce the required dose-response information for the missing crops in Table 6. Until such an experimental trial is conducted, the practical conclusion that can be drawn is not that the use of nano-micronutrients and PGR's in the field should be made indiscriminately, rather that a trial conducted on either the nano-micronutrient or the PGR alone should, at a minimum, report data on the PGR or nano-micronutrient: the number of nodules present and the index of hormonal application history for the nano-micronutrient trial, and the level of micronutrient sufficiency for the PGR trial. This will allow for the beginning of development of the combined dataset (presented in this review) needed to show both require complementary relationship to achieve an effect on plant productivity.

7. Conclusion

This review does not compare two overlapping literatures regarding whether there is strong evidence that there is true and greater than additive synergy between nano micronutrients and plant growth regulators in optimising the carbon nitrogen economy of grain legumes. Based on current evidence, the mechanistic synergies are strong, however there has not been direct empirical testing of these synergies. Nano micronutrients, especially nanoscale molybdenum, have been shown to increase the amount of nodule carbon investment converted to fixed nitrogen, primarily through the supply of cofactors and through delaying nodule senescence via antioxidant activity [9]. Plant Growth Regulators (PGRs) also control how much carbon accumulates in the nodule and how many nodules are there to use that carbon [16, 18, 20]. Based on the large costs associated with carbon allocations to the nodule(s) [27, 28], the effects of the two completely different synergies above should multiply rather than add when both functions are realised. In fact, the only combination trial known to the author that studied two PGRs supports that much of the mechanistic basis for synergy is through the adjacency of the pathways being investigated [15].

The prescribed and actionable research recommendation for researchers is no longer just 'involved tills combined more trials' as described in the general recommendation; rather, it is to undertake the factorial design suggested by Figure 3 and Proposition 1: whereby RNG treatments (i.e., nano-micronutrients) are applied simultaneously with PGRs in a grain-legume field trial with common fields measured on nodule production and nitrogenase activity, and, where applicable, the ^{13}C or ^{15}N tracer method for confirming the carbon allocation measured through both chemical-economics and experimental economics basis established in previous research (i.e. soybeans) are extended to include RED legumes (chickpea, mungbean and cowpea). Comparatively, through their ongoing analyses of these alternative applications of nano-PGRs, practitioners/policymakers could continue applying both nano-PGR seed treatments and foliar applications as efficacious interventions unto themselves, as well as their application where there is an identified constraint on nodule production and/or carbon allocations; however at the time there is currently no evidence to support formal guidelines for applying combined nano-PGRs because no trials have confected any of these two (cumulative) applications. In closing, the only actionable research recommendation to convert the central hypothesis of this review from hypothetical-mechanistic to agronomic-evidence-supporting is the preceding clearly particulars on the nexus of these two variables.

References

1. Campo RJ, Araujo RS, Hungria M. Importance of molybdenum and cobalt to the biological nitrogen fixation. In: *Nitrogen Fixation: From Molecules to Crop Productivity*. Dordrecht: Kluwer; 2000. p. 597–598.
2. Adhikari K, Missaoui AM. Molybdenum and boron nutrition and their roles in nitrogen fixation and yield of legumes. *Journal of Plant Nutrition*. 2017;40(15):2178–2190.
3. DeRosa MC, Monreal C, Schnitzer M, Walsh R, Sultan Y. Nanotechnology in fertilizers. *Nature Nanotechnology*. 2010;5(2):91–91.
4. Bhuvana J, Aravindhan R. Interaction between micronutrients and their effects on legume nodulation and nitrogen fixation. *International Journal of Plant and Soil Science*. 2023;35(18):45–56.
5. Rahman MM, Islam MT, Hasan MK. Synergistic effects of Rhizobium and micronutrients (molybdenum and boron) on the growth, nodulation, and yield of blackgram (Vigna mungo L.). *Journal of Soil and Plant Environment*. 2025;4(2):112–124.
6. Ullah A, Bano A, Khan N, Gaber A. Interactive effects of molybdenum, zinc and iron on the grain yield, quality, and nodulation of cowpea (Vigna unguiculata (L.) Walp.) in north-western India. *Sustainability*. 2022;14(11):6836–6836.
7. Naderi MR, Danesh-Shahraki A. Nanofertilizers and their roles in sustainable agriculture. *International Journal of Agricultural Science*. 2013;5(19):2229–2232.
8. Kumar N, Samota SR, Venkatesh K, Tripathi SC. Global trends in use of nano-fertilizers for crop production: advantages and constraints – a review. *Soil and Tillage Research*. 2023;228:105645–105645.
9. Li M, Zhang P, Guo Z, Cao W, Gao L, Li Y, *et al*. Molybdenum nanofertilizer boosts biological nitrogen fixation and yield of soybean through delaying nodule senescence and nutrition enhancement. *ACS Nano*. 2023;17(15):14761–14775.
10. Rizwan M, Ali S, Rehman MZU, Adrees M, Arshad M, Qayyum MF, *et al*. Bulk and nanoparticles of zinc oxide exerted their beneficial effects by conferring modifications in transcription factors, histone deacetylase, carbon and nitrogen assimilation, antioxidant biomarkers, and secondary metabolism in soybean. *Ecotoxicology and Environmental Safety*. 2021;222:112504–112504.
11. Palacio-Márquez A, Ramírez-Estrada CA, Gutiérrez-Ruelas NJ, Sánchez E, Ojeda-Barrios D, Chávez-Mendoza C. Efficiency of foliar application of zinc oxide nanoparticles versus zinc nitrate complexed with chitosan on nitrogen assimilation, photosynthetic activity, and production of green beans (Phaseolus vulgaris L.). *Plant Physiology and Biochemistry*. 2021;168:246–256.
12. Zhao L, Wang C, Deng B, Wang P, Zhou Q, Ma H, *et al*. Size effects of copper oxide nanoparticles on boosting soybean growth via differentially modulating nitrogen assimilation. *Environmental Science: Nano*. 2024;11(3):1122–1136.
13. Seleiman MF, Almutairi KF, Alotaibi M, Shami A, Alhammad BA, Battaglia ML. Zinc oxide and ferric oxide nanoparticles combination increase plant growth, yield, and quality of soybean under semiarid region. *Chemosphere*. 2024;352:141395–141395.
14. Ali EA, Mahmoud AM. Effect of foliar spray by different salicylic acid and zinc concentrations on seed yield and yield components of mungbean in sandy soil. *Asian Journal of Crop Science*. 2013;5(1):33–40.
15. Sun X, Liu Y, Chen W, Li Y, Shi G, Fan G, *et al*. Plant growth regulators improve nitrogen metabolism, yield, and quality of soybean–rhizobia symbiosis. *Annals of Microbiology*. 2023;73:14–14.
16. Boivin S, Fonouni-Farde C, Frugier F. How auxin and cytokinin phytohormones modulate root microbe symbioses. *Frontiers in Plant Science*. 2016;7:1240–1240.
17. Upreti KK, Murti GSR. Effects of brassinosteroids on growth, nodulation, phytohormone content and

nitrogenase activity in French bean under water stress. *Biological Plantarum*. 2004;48(3):407–411.
18. Jarzyniak KM, Guo X, Wang Z, Wang X, Kruszka K, Nowak K, *et al*. Local and systemic effect of cytokinins on soybean nodulation and regulation of their isopentenyl transferase (IPT) biosynthesis genes following rhizobia inoculation. *Journal of Experimental Botany*. 2021;72(20):6944–6959.
19. Ferguson BJ, Mens C, Hastwell AH, Zhang M, Su H, Jones CH, *et al*. Right time, right place: the dynamic role of hormones in rhizobial infection and nodulation of legumes. *Plant, Cell & Environment*. 2019;42(7):2077–2091.
20. van Zeijl A, Wardhani TAK, Seifi Kalhor M, Rutten L, Bu F, Hartog M, *et al*. CRISPR/Cas9-mediated mutagenesis of CLE peptides and NIN-LIKE PROTEINs and the autoregulation of nodulation pathway in *Medicago truncatula*. *Plant Cell Physiology*. 2020;61(2):340–352.
21. Sarraf M, Vishwakarma K, Kumar V, Arif N, Das S, Johnson R, *et al*. Synergistic interactions of nanoparticles and plant growth promoting rhizobacteria enhancing soil-plant systems: a multigenerational perspective. *Frontiers in Plant Science*. 2024;15:1376214–1376214.
22. Ceballos-Laita L, Perez-de-Luque A. Effectiveness of nanoformulated plant growth regulators in production and propagation of crops and ornamentals. *Philippine Journal of Science*. 2024;153(2):701–714.
23. Adhikari T, Kundu S, Rao AS. Seed priming with selenium and zinc nanoparticles modifies germination, growth, and yield of direct-seeded rice (Oryza sativa L.). *Scientific Reports*. 2022;12:5807–5807.
24. Rubia MI, Larrainzar E, Marino D. Drought stress modifies the source-sink dynamics of nitrogen-fixing soybean plants prioritizing roots and nodules. *Physiologia Plantarum*. 2025;177(3):e70276–e70276.
25. Udvardi M, Poole PS. Transport and metabolism in legume-rhizobia symbioses. *Annual Review of Plant Biology*. 2013;64:781–805.
26. Terpolilli JJ, Hood GA, Poole PS. What determines the efficiency of N2-fixing Rhizobium-legume symbioses? *Advances in Microbial Physiology*. 2012;60:325–389.
27. Minchin FR, Witty JF. Respiratory/carbon costs of symbiotic nitrogen fixation in legumes. In: *Plant Respiration*. Dordrecht: Springer; 2005. p. 195–205.
28. Voisin AS, Salon C. Nodule formation and functioning in relation to root nodule bacteria: carbon and nitrogen relationships in the legume nodule. In: *Nitrogen Fixation in Crop Production*. Madison: ASA-CSSA-SSSA; 2010. p. 21–47.
29. Julkowska MM, Testerink C. Tuning plant signaling and growth to survive salt. *Trends in Plant Science*. 2015;20(9):586–594.
30. Sulieman S, Tran LSP. Symbiotic nitrogen fixation legume genome and microbiome interactions. *Frontiers in Plant Science*. 2016;7:1620–1620.
31. Kohlen W, Charnikhova T, Lammers M, Pollina T, Toth P, Haider I, *et al*. The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SlCCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. *New Phytologist*. 2012;196(2):535–547.
32. Ferguson BJ, Lin MH, Gresshoff PM. Regulation of legume nodulation by acidic growth conditions. *Plant Signaling & Behavior*. 2013;8(3):e23426–e23426.
33. Goyal RK, Mattoo AK, Schmidt MA. Rhizobial-host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability. *Frontiers in Microbiology*. 2021;12:669404–669404.
34. Hossain A, Quddus MA, Alam MK, Naser HM, Anwar B, Khatun MF, *et al*. Application of zinc, boron, and molybdenum in soil increases lentil productivity, nutrient uptake, and apparent balance. *Canadian Journal of Soil Science*. 2021;101(1):113–124.
35. McIntyre KE, Bush DR, Argueso CT. Cytokinin regulation of source-sink relationships in plant-pathogen interactions. *Frontiers in Plant Science*. 2021;12:677585–677585.