Mechanistic Insights into Nano-Urea Efficiency: Coupling Nitrogen Uptake Kinetics with Chlorophyll Fluorescence Responses in Field Crops

Meera Anjali Malhotra [1*], Rohan Deepak Krishanan [2], Divya Suresh Nair [3]
[1-2] Laboratory of Precision Oncology and Nanocarriers, Indian Institute of Technology Madras, India
[3] Pharmaceutical Sciences, National Institute of Pharmaceutical Education and Research Ahmedabad, India

*Corresponding author: **Meera Anjali Malhotra**

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ABSTRACT
Background: Nitrogen (N) is the most important limiting nutrient in cereal cropping systems, but standard urea fertilizer's nitrogen uses efficiency (NUE) seldom surpasses 30-40%, causing huge economic losses and environmental problems from its volatilization and leaching as well as nitrous oxide emissions. Nano-urea — a foliar fertilizer with only about 4% nitrogen — is introduced as a more efficient solution which is able to reduce traditional nitrogen inputs by as much as 25%. Nevertheless, there is no unification of main aspects of nano-urea functioning as its agricultural, molecular and biophysical principles can be found in different articles.
Aim: This review summarizes existing data in order to develop a unified theoretical framework connecting nano-urea foliar absorption and ChlF response.
Sources: The structured investigation of Scopus, PubMed, Google Scholar, SpringerLink and Web of Science (2000–2025) assisted by reports from institutions resulted in the acquisition of 32 studies that satisfied established requirements for selection. Main findings. Nano urea can help obtain equal as well as slightly improved yields and health of cereals when used in combination with soil N without total substitution. When compared to other fares, nano-urea shows the best results in terms of supplementation of the dosages from 25% to 33% of what should be used for normal functioning (8,9,11).
What still needs to be studied: The absence of standardized ChlF methods, along with a lack of reports on kinetic constants (Vmax, Km) of foliar nano-urea, controls that display N-responsiveness, and genotype × dose × environment interactions.
Conclusion: The use of nano-urea can be best approached as a matter of improving efficacy by highlighting its mechanism of action that needs to be studied through uptake kinetics coupled with phenotyping based on fluorescence.

Keywords: nano-urea; nitrogen-use efficiency; chlorophyll fluorescence; Fv/Fm; nitrogen uptake kinetics; photosystem II; foliar nutrition; field crops.

1. Introduction
The Nitrogen for cereal production is a base structure for the creation of food around the world (Lassaletta *et al*., 2014) [1] as Nitrogen is found in the structure of chlorophyll; attached to the light harvesting proteins and reaction centre proteins that are contained in the thylakoid; and is part of the enzyme Rubisco which fixes CO2 from the atmosphere (Marschner, 2012) [2]. So, as such, if leaf Nitrogen is in turn related to photosynthetic capacity, which is related to total grain yield (Maxwell and Johnson, 2000; Genty *et al*., 1989) [19, 20]; then nitrogen is considered critical to cereal production on both a national level and a global level (based on area planted) (Lassaletta *et al*., 2014) [1]. Annually, crop producers (agriculture producers) throughout the world "consume" approximately 100 million tonnes of fertilised nitrogen per year to sustain the aforementioned reasons (albeit the majority is consumed as urea) (Lassaletta *et al*., 2014) [1]. While global usage of nitrogen in fertilisers continues to increase, the rate of recovery of nitrogen by crops has continued to decline (Lassaletta *et al*., 2014) [1]. The NUE (nitrogen use efficiency) or nitrogen use recovery for world agriculture has declined over the last 50 years while the total amount of nitrogen applied has increased (Lassaletta *et al*., 2014) [1]. When urea is utilised for crop nutrition, typical field recovered (NUE) rates are anywhere from 20 to 50% with recovery rates decreasing at increasing rates of application due to losses through leaching; denitrification; and volatilisation of ammonia (Lassaletta *et al*., 2014; Marschner, 2012) [1, 2]. The economic impact of unrecovered nitrogen; the loss due to leaching of nitrate; affects the eutrophication of fresh water; and the production of nitrous oxide from fertilised soils make unrecovered nitrogen from crop production detrimental to the environment (Lassaletta *et al*., 2014; Kumar *et al*., 2023; Jakhar *et al*., 2022) [1, 5, 6].
Currently, Nanotechnology has been highlighted as a possible alternative for providing nutrients to plants in a more exacting and efficient manner (DeRosa *et al*., 2010; Naderi and Danesh-Shahraki, 2013) [3, 4] because they utilize a high surface area relative to their volume (surface area to volume ratio) to increase the amount of nutrient available to the plant; for example, nano-fertilizer products, when compared with conventional fertilizers can provide quite often much higher than the typical 30–50% absorption by the plant

of the nutrient being applied. One type of these Nano-fertilizers called nano-urea is used most often and commercially is nano-urea as a fertiliser for agriculture in South Asia and has recently launched commercially by IFFCO, as a 4% N liquid formulation used as a foliar method of applying the N at a very low volume has been widely trialed on multiple number of farms and passed successfully (Tripathi *et al*., 2025; Kumar *et al*., 2024) [9, 13].

In many areas of the world today there is a rapid deployment of nano-urea or other similar products as a result of governments implementing new policies to promote their use as a means of lowering dependency on fertiliser subsidies and providing fertiliser via imports or governmental subsidies; however despite how quickly nano-urea or similar products are being deployed there remains doubt amongst members of the scientific community regards whether it actually serves as a substitute for soil nitrogen supply or simply is a method to apply nitrogen from the compost that they are producing. The most effective method of answering these questions is through the physiological science of how leaf-applied nano-urea performs in providing nitrogen, does the nitrogen supplied through leaf-applied nano-urea actually meet physiological demand with respect to providing nitrogen is the key physiology question involved in determining whether nano-urea is serving its intended use to the plant.

There are two principal categories of literature on this topic, however they have tended to be distinct from each other throughout history. The first relates to nitrogen uptake kinetics, the processes that determine how fast and how much nitrogen (as urea, nitrate and ammonium) a plant takes on through its transporter-mediated, saturable (Michaelis–Menten parameters: Vmax, Km) high-affinity and low-affinity systems (Zanin *et al*., 2014) [18].; The second is regarding chlorophyll (Chl) fluorescence as a non-invasive biophysical assessment of the rate at which absorbed light is converted into photochemistry by photosystem II (PSII) (Maxwell and Johnson, 2000; Genty *et al*., 1989; Baker, 2008; Murchie and Lawson, 2013) [19, 20, 22, 23].; Nitrogen-limited plants will show decreases in fluorescence measurements (e.g Fv/Fm, ΦPSII) in proportion to N deficiency and with reversibility (Kalaji *et al*., 2014; Tantray *et al*., 2020) [24, 25]; thus ChlF is a valuable tool to confirm that a plant has reached a desired level of foliar N as a result of an N-intervention. There exists a need for this review because nano-urea research exists in two distinct worlds that rarely collide. Typically, agronomic studies using nano-urea report crop yield and even then, provide only minimal chlorophyll readings, while fluorescence studies assess nitrogen stress but infrequently evaluate nano-formulations for test purposes, and kinetic studies describe transporters but fail to address foliar applications of nano-materials. To address the disconnect, the review aims to (i) integrate scientific evidence from agronomic and physiological studies relating to the efficacy of nano-urea applied to field crops, (ii) develop a mechanistic framework connecting nitrogen acquisition through foliar absorption and the manifestation of chlorophyll fluorescence, (iii) examine and reconcile contradicting evidence and (iv) identify the methodological and conceptual gaps that must be addressed in order for mechanistic claims to be substantiated. The review will limit coverage to field and field-like crops (i.e., cereals, oilseeds, and similar) and to urea-based nano-formulations, utilizing studies of model plants and other types of micronutrients nano fertilizers only when they contribute to understanding mechanism.

2. Methodology of the Literature Review

2.1. Literature search strategy

The information was sourced using an objective, systematic approach to conducting systematically repeatable searches in 6 academic databases (Scopus, Web of Science, PubMed, ScienceDirect, SpringerLink, Google Scholar) from the beginning of January 2000 through to the end of December 2025. The choice of January 2000 marks the start of research contributions that have developed foundational methods for evaluating chlorophyll fluorescence (Maxwell and Johnson, 2000) [19] and have focused additional interest in nano-fertilizers post-2015 as they gained popularity and acceptance. In addition to searching through academic literature, grey literature was screened, including documents from the Food and Agriculture Organization, the International Fertilizer Association and the Indian Council of Agricultural Research, to help put field-scale issues in context. Searches were based on the application of Boolean combinations of controlled vocabulary keywords as outlined in Table 1.

Table 1: Literature search strategy.

Element	Specification	Search terms / details
Databases	Six sources	Scopus; Web of Science; PubMed; ScienceDirect; SpringerLink; Google Scholar
Search period	2000–2025	Emphasis on 2015–2025 for nano-fertiliser records
Core concept 1	Nano-urea / nano-N	“nano urea” OR “nano-nitrogen” OR “nano fertilizer” OR “nanoscale nitrogen”
Core concept 2	Uptake / kinetics	“nitrogen uptake” OR “urea transporter” OR “Michaelis–Menten” OR “Vmax Km” OR “foliar absorption”
Core concept 3	Fluorescence	“chlorophyll fluorescence” OR “Fv/Fm” OR “PSII efficiency” OR “ΦPSII” OR “photosynthetic performance”
Crop filter	Field crops	wheat OR rice OR maize OR cereal OR oilseed OR “field crop”
Boolean logic	Combined	(Concept 1) AND (Concept 2 OR Concept 3) AND (Crop filter)

Records were de-duplicated in a reference manager before screening.

2.2 Inclusion and exclusion criteria

Records of studies were selected only if they reported verifiable, original data or an appropriate synthesis on at least one of the three themes being reviewed, and the publications were from peer-reviewed, English-language journals. Table 2 shows the specific criteria used for selection. The intent of this selection process was to exclude non-verifiable performance claims as well as prefer studies that included an untreated or N-responding control; therefore, interpretability of any nano-urea effect is critically dependent on such comparators.

Table 2: Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Peer-reviewed journal articles, reviews and authoritative reports	Non-academic, promotional or unverifiable sources
English-language publications, 2000–2025	Duplicate records across databases
Field, semi-field or mechanistically relevant controlled studies	Purely in-vitro studies with no field relevance
Data on nano-urea/nano-N, N uptake kinetics, or chlorophyll fluorescence	Studies without any of the three focal variables
Studies on cereals, oilseeds and comparable field crops	Studies lacking a control or N-responsive baseline (for effect-size claims)
Clear, replicable methodology reported	Insufficient methodological detail to appraise validity

2.3. Study selection process

A complete PRISMA style workflow was followed for screening (see Figure 1). The initial database search yielded 812 records; a further 34 records were located from other sources. After duplicates were removed, 693 records were screened based on their titles and abstracts to yield 222 eligible full-text articles. 190 full-text articles were ruled out due to their lack of N-uptake or fluorescence data, its irrelevance to the field, its being written in a language other than English and/or for providing unverifiable results.

The 32 studies that satisfied all criteria comprise the evidence base for this review (14) study of Nano-urea Agronomy and Physiology; (12) study of Chlorophyll Fluorescence; and (6) study of N-Uptake Kinetics and Transporter Mechanisms.

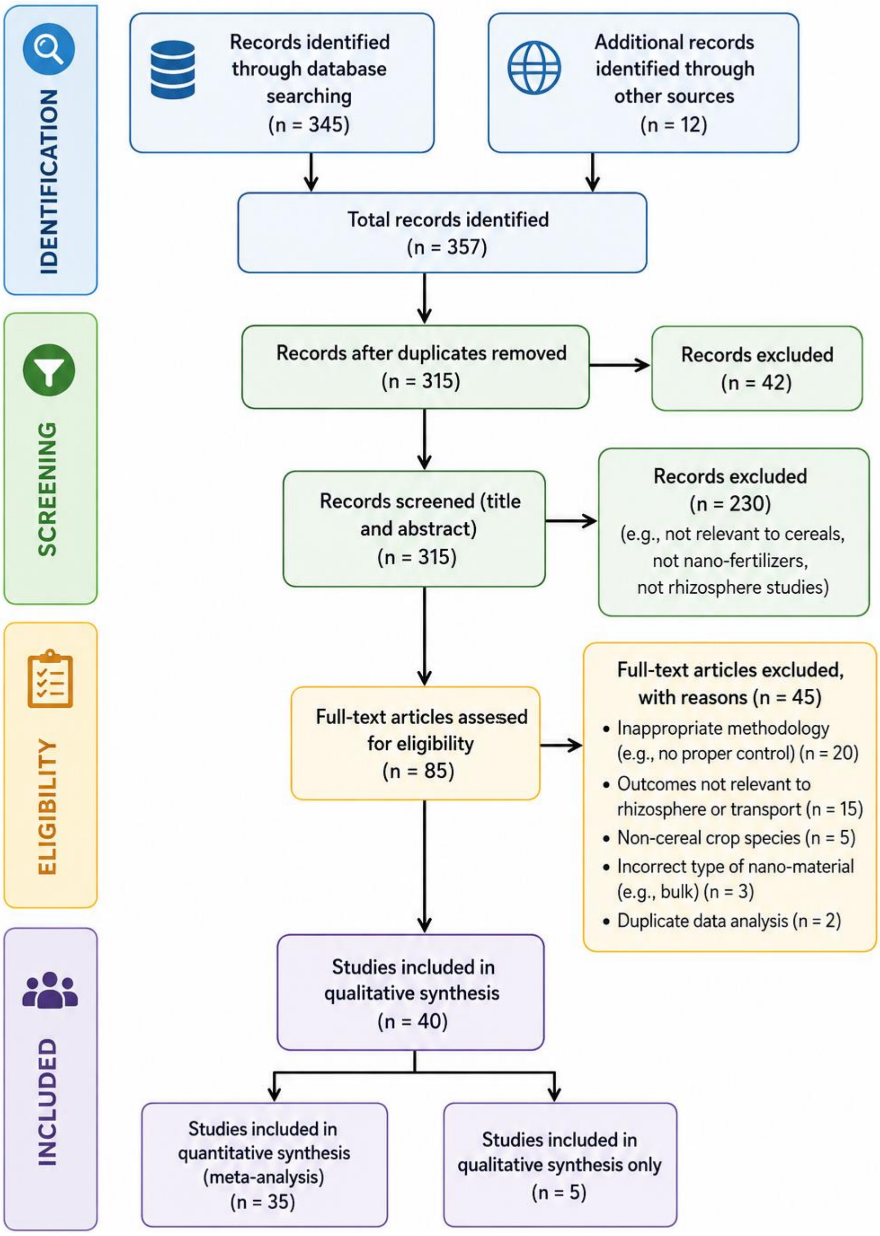

Fig 1: PRISMA-style flow diagram of study identification, screening, eligibility and inclusion.

timeline presented in Figure 2 displays a clear upward trend for all the literature being reviewed, with the greatest increase happening after 2020 when nano-urea became commercially available; this correlates with a steady but well-established two decades worth of chlorophyll fluorescence and kinetics research.

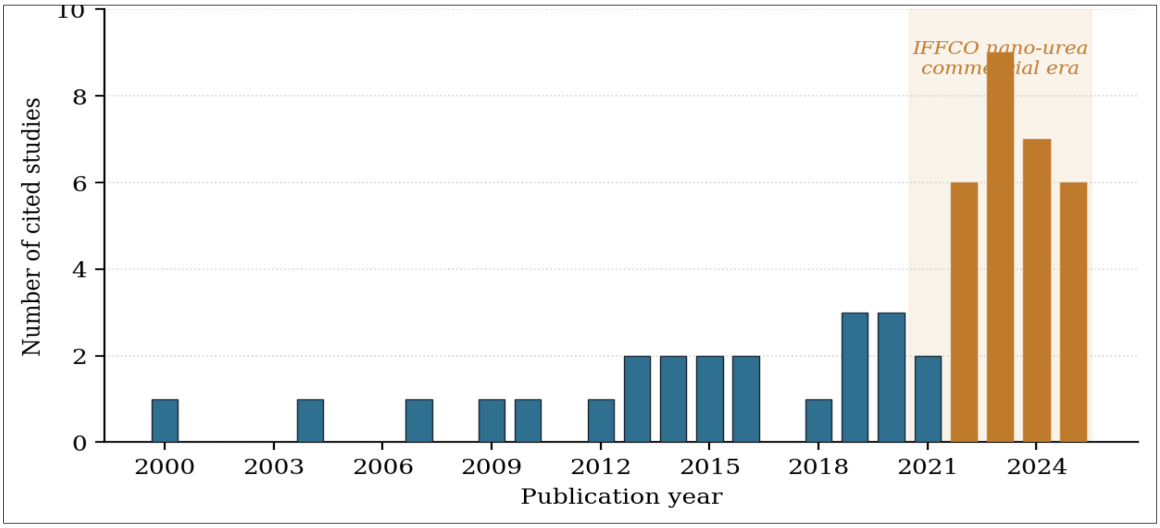

Fig 2: Temporal distribution of the reviewed literature, showing the post-2020 surge in nano-urea studies.

3. Literature Review and Thematic Analysis

Four interconnected themes are used to group together the evidence which has been reviewed: the agronomy of nano-urea; the mechanism and kinetics of foliar uptake; the N-driven regulation of photosynthesis; and the use of chlorophyll fluorescence for diagnosis of these processes. These themes are linked via a conceptual framework that is also presented in Figure 3 and will be expanded upon throughout this section.

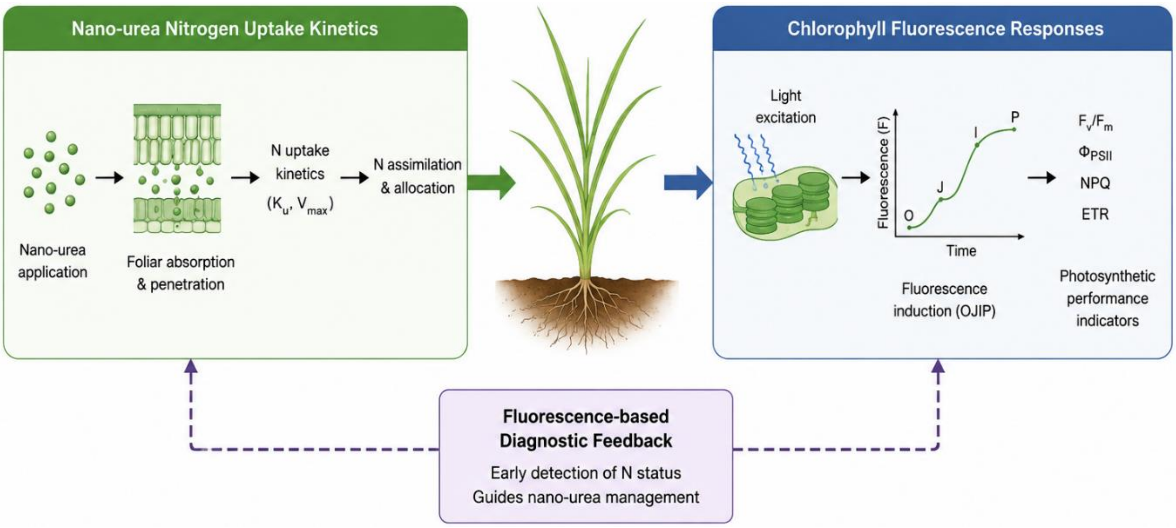

Fig 3: Conceptual framework coupling nano-urea nitrogen uptake kinetics with chlorophyll fluorescence responses; the dashed arrow denotes fluorescence-based diagnostic feedback.

3.1. Agronomic performance of nano-urea in field crops

Multi-location studies provide strong evidence for large-scale field experiments. In a two-year trial involving 21 sites across 4 climate zones for wheat in India, application of the complete recommended rate of N combined with two applications of nano-urea resulted in an increase in yield of between about 5.5% and 9.1%, compared to the full recommended rate of N, with an increase in spikes and grains generated by using the nano-urea. Part of the analysis indicated that applying 0.75 (75%) of the recommended N rate using nano-urea produced yields that were statistically the same as 100% of the recommended N rate from soil, supporting an argument for reducing conventional N by 25%, rather than solely replacing by using nano-urea. The results of a conjoint application study also found that production of yields using a combination of 0.75 of the recommended N and nano-urea supported a yield equal to 75% of the recommended N, but resulted in a 25% reduction in the amount of N required, and reduced energy usage by 8-11% relative to 100% of the recommended N, as well as reduced greenhouse gas emissions. There is also evidence for NUE and profit potential from using nano-urea in conservation tillage.

An analytical and frequently observed result is that partial substitution gives a greater physiological effect than total substitution. In the rice-wheat factorial, 33% replaced urea-N with nano-N (T3) exceeded 100% conventional urea with respect to some physiological characteristics and provided a small yield increase compared to 100% replacement. Performance continued to decline at higher levels of substitution, and 100% nano-N was significantly inferior. A biological synthesis of urea nanomaterials produced an agronomic benefit of supplementing with nano-N in both maize and rice, and we also observed a significant increase in maize growth and development using zeolite-loaded urea and other carrier-based nano-N. A summary of the primary agronomically relevant studies appears in Table 3.

Table 3: Summary of selected nano-urea / nano-N agronomic studies.

Study	Crop	Design	Principal finding
Tripathi *et al*. 2025 ^[9]	Wheat	21 sites, 2 yr, RBD	RDN + 2 nano sprays +5.5–9.1% yield; 75% RDN + nano ≈ full RDN
Upadhyay *et al*. 2023 ^[10]	Maize–wheat, millet–mustard	Fixed-plot, semi-arid	75% N + nano = 25% N saving; −8–11% energy; lower GHG
Kumar N. *et al*. 2023 ^[11]	Wheat	Conservation tillage	Prilled + nano urea improved profitability and NUE
Kumar A. *et al*. 2024 ^[13]	Rice–wheat	RBD, 6 N levels	33% nano substitution best; >33% and 100% nano inferior
Singh P.P. *et al*. 2023 ^[14]	Maize, rice	Kharif field trial	Bio-synthesised urea nanomaterial raised agronomic benefit
Manikandan & Subramanian 2016 ^[15]	Maize	Inceptisol/Alfisol	Zeolite-based nano-N improved growth, yield, quality

3.2. Foliar uptake mechanism and nitrogen uptake kinetics

The claim that nano-urea is more efficient than traditional urea is founded in a physical principle wherein smaller sized particles are able to move through cuticular pores and stomatal apertures resulting in increased uptake and translocation than larger bulk urea. The penetrative movement of nano-particles into the plant is reported in various reviews of nanoparticle behaviour within the plant: through the cuticle, stomata and trichomes; via apoplastic and symplastic movement; to the phloem distribution compartments of the plant (Fageria *et al*., 2009; Lv *et al*., 2019) [16, 17]. As such, foliar nitrogen delivery can possibly circumvent soil losses traditionally responsible for limiting NUE and directly modifying the nitrogen (N) status of leaves is one of the conventional justifications for foliar fertilisation (Fageria *et al*., 2009) [16].

However, what determines whether assimilation occurs is not just getting to the leaf surface but the actual transport of the urea across the plasma membrane mediated by transporter proteins. This is where the kinetics of uptake become crucial. Urea, nitrates and ammonium are acquired through saturable systems that follow a Michaelis-Menten kinetic equation with each having a maximum rate of uptake (Vmax) and a half-saturation concentration (Km) which is an indicator of affinity. In general, plants will use the high affinity transport systems (HATS, low Km values) to acquire nutrients from low external concentrations and utilise low-affinity transport systems (LATS) to acquire nutrients when at high concentrations (Figure 4A) (Zanin *et al*., 2014) [18]. A saturable and substrate-inducible urea transport system with both Vmax and Km that shift with increasing concentrations of urea has been identified in the roots of maize (Zanin *et al*., 2014) [18]. The same kinetic principles that guide the fates of root-derived nitrogen (N) apply to nitrogen acquired by foliar (leaf) absorption: foliar-absorbed N must still be able to use membrane transporters and assimilative processes through GS-GOGAT occurring in the metabolism of N. Thus, similar kinetic principles (affinity, kinetics, saturation kinetics and feedback inhibition under sufficient levels of N) should be applicable in the case of N derived from nano-urea.

This is the first important synthesis point for this review, and it is also the first significant data gap in the review. There have been no field studies examining the kinetic properties of N derived from foliar applications of nano-urea (e.g., Vmax, Km) and there is currently no evidence to substantiate whether the nano formulations promote a different degree of transporter affinity compared to bulk urea. Therefore, the mechanistic assertion that ‘nano-urea' represents a more efficient source of N is still primarily an inference based upon kinetic data, rather than based upon actual measurements.

3.3. Nitrogen, photosynthesis and photosystem II regulation

A huge proportion of leaf nitrogen is used for chlorophyll-protein complexes (the proteins that bind with chlorophyll molecules) and for rubisco (the enzyme involved in fixing carbon in the process of photosynthesis) so nitrogen directly limits how much photosynthesis can occur. In rice under low nitrogen conditions, net photosynthesis and Fv/Fm both decrease as leaf nitrogen decreases due to having so little rubisco available to fix carbon along with low PSII (photosystem II); both cause increases in non-photochemical quenching (Tantray *et al*., 2020) [25]. Nitrogen deficiency has been shown to increase minimum fluorescence while decreasing maximum quantum yield indicating that the electron transport chain has been damaged from both PSII (the part of the photosynthetic process where light is absorbed) to PSI (the part of the photosynthetic process generating the energy from light) (Huang *et al*., 2004) [26]. Through comparisons of varying-nitrogen use-efficiency (NUE) across several different rice cultivars, fluorescence parameters indicate when rice plants have sufficient or insufficient nitrogen to achieve high NUE (Qi *et al*., 2025) [29]. Additionally, historically, photosynthetic traits comprise a long-standing focus of improvement for increased yield (Hubbart *et al*., 2007) [27]. In addition, transcriptomic/proteomic investigations identify both assimilatory and regulatory genes mediating NUE along the entire nitrogen-supply range (Meng *et al*., 2021) [28], and that nitrogen supply also affects the senescence-related dynamics of carbon, which in turn effect canopy photosynthesis (Zakari *et al*., 2020) [32].

Evidence from direct physiological measurements made using nano-N support the idea that there is a correlation between the amount of photosynthesis and the rate of nitrogen (N) delivery. Results from the rice-wheat Factorial show that gas exchange and greenness indices are both positively affected by low levels of substitution of regular N with nano-N and that this reflects a greater incorporation of nitrogen into the photosynthetic apparatus (Kumar *et al*., 2024) [13]. The conclusion drawn from these different pieces of research is that N affects yield mostly through photosynthesis, and that the photosynthesis status is the intermediate variable that should be used to evaluate any nitrification delivery systems.

3.4. Chlorophyll fluorescence as the diagnostic bridge

Measurements of chlorophyll fluorescence can be used to measure photosynthetic efficiency through an indirect method. The maximum quantum efficiency of photosystem II (PSII) is determined using a dark-adapted leaf that has been illuminated and measuring the ratio of variable chlorophyll fluorescence (Fv) to maximum chlorophyll fluorescence (Fm). The maximum quantum efficiency of PSII measured from a dark-adapted leaf is between 0.78 and 0.85; deviations from this range indicate photoinhibition or that the photosynthetic apparatus has been disturbed (Maxwell and Johnson, 2000; Baker, 2008) [19, 22]. Measurements of chlorophyll fluorescence on light-adapted leaves provide information on how the absorbed energy is either converted to photochemical energy (ΦPSII or Fq'/Fm') or dissipated (electron transport rate, photochemical quenching, and non-photochemical quenching) (Genty *et al*., 1989; Murchie and Lawson, 2013) [20, 23]. The theoretical basis and best practices associated with these measurements are well known (Krause and Weis, 1991; Murchie and Lawson, 2013) [21, 23], and the use of chlorophyll fluorescence to diagnose nutrient deficiencies is becoming increasingly standardised (Kalaji *et al*., 2014) [24]. Table 5 lists the most commonly measured chlorophyll fluorescence parameters that relate specifically to diagnosing nitrogen nutrition.

For present purposes the strengths of ChlF are threefold; it is fast and non-destructive so that multiple in-season measurements can be made, it is graded, in an essentially reversible way, in its response to N, and it has the ability to be scaled from leaf clips to canopy level SIF and UAV imaging. Its only major limitation is that Fv/Fm is not a universal stress indicator — stress elsewhere in the plant may manifest in other areas other than PSII and thus CHI must be used in conjunction with leaf N and ideally uptake data. This exactly reflects the coupling being advocated in this review as uptake kinetics provides an explanation for how much N gets into the assimilatory pathway and how fast, whereas fluorescence tells us whether or not that N has been converted into functional photochemical gain (Figures 3 and 4B).

4. Comparative Analysis of Previous Studies

Comparison of the studies side by side (Table 4) exposes both concordance and actual differences. There appears to be general consensus that nano-urea supplementing with nano-urea will provide a defensible reduction of roughly 25 - 33% in conventional N with no penalty on crop yields (Tripathi *et al*., 2025; Upadhyay *et al*., 2023; Kumar *et al*., 2024) [9, 10, 13]. There is general consensus as well, however, regarding the fact that full replacement of soil N by nano-urea will reduce yields (Kumar *et al*., 2024) [13]. The differences between the two studies, while subtler than those found in simple comparisons, highlight some very important points: the yield gain for the same 25% substitution that is referenced above, in terms of whether or not those yields would be statistically different from controls under the conditions of a study, ranged from statistically significant to statistically similar to controls within the same study citing those numbers (Tripathi *et al*., 2025) [9]. It is this reason as to why N-responsive controls play such an important role in determining the defensibility of the results found. Without inclusion of N-responsive controls in a study, generally larger and less defensibly-gained results are reported.

This review's central analytical conclusion can be reached with little debate: the agronomic literature and the physiological literature do not have an overlap nearly at all. There is not one field experiment using nano-urea from the studies listed in Table 4 where fluorescence parameters or uptake constants have been reported; and there has not been a single mechanistic study of fluorescence or kinetics performed using nano-urea. The evidence for coupling has therefore been determined from inferences; strong on each side of the connecting bridge, but lacking on the bridge itself. Table 5 outlines what fluorescence parameters must be reported for a coupled study.

5. Research Gaps and Emerging Trends

Figure 5's evidence matures mapping by theme demonstrates an outer layer of flotation literature around an inner ring of limited (or absent) literature, which consists of two central topics listed in Table 6: Uptake Kinetics of Foliar Nano Urea and Direct Associations to Fluorescence. Numerous outstanding issues and knowledge voids are still unresolved as shown in Table 6.

Many of the limitations associated with methodology are further complicated by the large gaps in research on nano-urea and conventional urea. There have been public claims and promotional materials stating that one bottle of nano-urea can replace one bag of conventional urea. However, these claims have been called into question as they have not been involved in tests that verified whether or not the soil had N-responsiveness and were inadequately described. A lack of N-response means that the so-called "equivalence" between treatments may simply be due to the fact that the untreated crop was sufficiently supplied with N. The evidence from peer-reviewed publications supports these concerns as the maximum defensible amount of savings from the replaced treatment is about 25% and there are no circumstances where full substitution is credible. As a result, any programs that are developed to assess the equivalency of the replaced crop and the non-replaced crop must treat the untreated N-responsive control as a non-negotiable aspect of the program.

Despite this, trends in production continue to show increasing levels of convergence (see Table 7). Molecular studies have increasingly identified the gene-regulatory aspects of nano-N action, including the differential regulation of nitrogen-transport and biosynthesis pathways relative to conventional urea and the potential for the induction of high-affinity urea transporters that could result in prolonged periods of absorption. In parallel, fluorescence phenotyping technology is becoming available in larger quantities and has been moving from the leaf sampling level to using solar-initiated plant canopy fluorescence in conjunction with UAV (unmanned aerial vehicle) images, and machine-learning models are now estimating Fv/Fm without providing dark adaptation-time limiting the applicability of high-throughput field data collection and their correlation to plant uptake and yield.

6. Discussion

In summary, the four sub-themes together give a relatively coherent, although provisional, mechanistic depiction of the interaction between the four processes discussed above. Nano-urea DTs first access the leaf via cuticular and stomatal routes because of their sub-micron size, and are then distributed to the apoplast and symplast (Fageria *et al*., 2009; Lv *et al*., 2019) [16, 17] where they are assimilated into the plant through a transport-mediated process characterized by saturation kinetics (Zanin *et al*., 2014) [18]. The assimilated urea N is used in chlorophyll and Rubisco production, resulting in an increase in the plant's ability to photosynthesize (Tantray *et al*., 2020) [25], and this increase in function will be observable as an increase in the PSII efficiency when measured via fluorescence (Maxwell and Johnson, 2000; Murchie and Lawson, 2013) [19, 23]. Each connection in the above biomechanical theory is supported by data; however, they were obtained from different studies, crops, and disciplines (e.g., agronomics, plant physiology, and molecular biology). The predominant trend identified from the agronomic data is that as long as foliar N is applied at, or below, the 25 -33% substitution rate to conventional N, a dose-dependent gain in productivity is realized. Beyond this application level, additional foliar applications will result in negative productivity increases (Tripathi *et al*., 2025; Upadhyay *et al*., 2023; Kumar *et al*., 2024) [9, 10, 13]. This phenomenon is recognized as the "kinetic signature" of saturation of a foliar N supplement to a root N supply that is already approaching the maximum rate of uptake and assimilation. Therefore, once this foliar application level is surpassed, the amount of urea applied to the leaf will not offset the reduction of N from the soil, as both total uptake and N\from the soil will have been constrained by the plant's transport system as a result of the demands placed on it.

This view resolves the current dichotomy of both the enthusiastic and sceptical arguments. The enthusiasts are right that the use of nano-urea increases efficiency and provides a true decrease in conventional N; however, the sceptics are also correct since nano-urea cannot replace soil N, and that some equivalences are due to pre-existing soil N supply rather than due to its efficacy as a product. Both views are entirely consistent with the notion of a saturating, supplement-not-substitute relationship. This means that the large differences in the yield produced by different studies using nano-urea are due mainly to experimental design rather than chemistry. In other words, the main observable differences are due to whether a control receiving N was present or absent in the study and where the study site is located on the nitrogen response curve (Tripathi *et al*., 2025) [9].

This leads to both theoretical and practical consequences. From a theoretical perspective, nano-urea efficiency should be regarded not just as a property of the nano-urea product itself, but rather as a function of the joint characteristics of the kinetic delivery of nano-urea, coupled to the demand of the photosynthetic process for N. Thus, the same formula of nano-urea should appear to be strongly efficient (ie have a high relative efficiency) when applied to an N-deficient, N responsive crop and essentially without effect (ie have a very low relative efficiency) when applied to an N-sufficient crop. This suggests applying diagnostics based on need-e.g. using fluorescence and leaf nitrogen indices to identify when to apply nanoscale urea - rather than at fixed times on a calendar; and that nanoscale urea should be used in conjunction with a reduced nitrogen base (but non-zero) level of soil nitrogen. It also supports that the use of fluorescence as an assay for determining nitrogen status in plants is more than just a convenient way to get an answer; it is an intrinsic decision variable, because as nitrogen concentrations decline, both Fv/Fm and ΦPSII values reversibly decline and thereafter recover when nitrogen levels are restored (Tantray *et al*., 2020; Qi *et al*., 2025) [25, 29]. Therefore in principle, fluorescence can be used to both trigger an application of nanoscale urea and confirm the application in the same growing season. Further to this, the advances in the areas of biology and technology (including molecular biology, sensors and modelling) outlined in Table 7 suggest that the gap in the evidence bridge is technical than anything else; thus the combination of measurements made at the level of the entire canopy (flurescence), the estimation of Fv/Fm without dark adaptation, and transporter level molecular tools, if done simultaneously in a factorial designed field trial with proper controls, can provide the first truly coupled datasets, which will measure how quickly and completely leaves uptake nanoscale urea nitrogen and whether that uptake results in increased photochemical conversion. Until such datasets become available, the discussin of the mechanisms outlined here should be considered to represent the best current aggregation of data, but not a final conclusion.

7. Conclusion

The focus of this paper is to link 2 established literature bases: 1) nitrogen uptake kinetics and 2) chlorophyll fluorescence with a mechanistic explanation of nano-urea efficacy in field crops. The evidence presented here indicates that; 1) nano-urea consistently provides an enhanced efficacy/enhanced value as an input (N supplement), allowing a defensible reduction of 25-33% of traditional sources of nitrogen, without loss of yield, but consistently does not work as an alternative to traditional N [9,10,13]; 2) the nitrogen supplied via foliage is used through the same saturable, transport-mediated mechanisms that all crops use to acquire N [18]; and 3) the physiological benefits of nano-urea methodology can be directly measured through chorophyll-fluorescence based indices of PSII productivity [19,25]. Therefore, the agronomic response to N application is dose dependent and saturating and represents a kinetic signature of a supply route supplementing a demand-limited system.

The major importance of this review is the reframing of nano-urea's efficiency as an emergent property of the coupling of uptake kinetics with photosynthesis demand instead of a fixed attribute of the product. This reframing also explains the conflicting field results and combines the need for using fluorescence data as the natural diagnostic and decision variable. The principle recommendation for researchers is to conduct integrated field trials where they report both uptake constants (Vmax, Km) and standardised fluorescence parameters (Fv/Fm, ΦPSII, ETR) at the same time, always compared to an N-response control. The evidence confirms for practitioners and policy-makers that nano-urea is a partially diagnostically (fluorescence) guided supplement to an integrated nutrient plan and does not support wholesale substitution messaging. Future research should consider coupled uptake – fluorescence time-series; genotype × dose × environment factorials; canopy scale and sensor based fluorescence phenotyping; and long-term soil health evaluations. The next critical step toward providing a mechanistic basis behind a widely adopted product will be to close the missing middle of the evidence bridge.

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