HIGHLIGHTS

- Current practice for managing nitrogen (N) use for cereal production are not environmentally sustainable. Over-use of N fertilizers is a global problem for millions of farmers who must decide on N applications- whether, when and how much.
- A combination of improved advice on N management for specific cropping regimes is required, together with a breeding target of new commercial crop varieties with sustainable yields and a low N requirement.
- While N use efficiency (NUE) has been a useful concept for quantifying the genetic differences in N uptake and utilization, the concept of an economic N optimum derived from N yield dose-response curves may provide new insights for lowering the N requirement

A roadmap for lowering crop nitrogen requirement

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Abstract

Increasing nitrogen fertilizer applications have sustained a growing world population in the 20th century. However, to avoid any further associated environmental damage, new sustainable agronomic practices together with new cultivars must be developed. To date the concept of nitrogen use efficiency has been useful in quantifying the processes of nitrogen uptake and utilization but we propose a shift in focus to consider nitrogen responsiveness as a more appropriate trait to select varieties with lower nitrogen requirements. We provide a roadmap to integrate regulation of nitrogen uptake and assimilation into varietal selection and crop breeding programs. The overall goal is to reduce nitrogen inputs by farmers growing crops in contrasting cropping systems around the world, whilst sustaining yields and reducing greenhouse gas emissions.

The global nitrogen challenge

The demand of a growing world population requires an increased food supply with a lower environmental footprint. In the 20th century, the population growth was sustained by increased crop yield resulting mainly from the production and application of synthetic nitrogen (N) fertilizer together with the selection of modern crop varieties. Current practices in N fertilizer use for crop production are not sustainable.

The global rise in N fertilizer consumption (increased from 11.3 Tg N yr^{-1} in 1961 to 107.6 Tg N yr^{-1} in 2013 [1]), together with the enhanced cropping of legumes that establish symbiotic interactions with N_2 fixing bacteria, has expanded the pool of reactive N in the environment [2,3], with significant environmental consequences. High amounts of greenhouse gas (GHG) emissions have occurred either directly through fertilizer production or indirectly via fertilizer-related losses as N_2O (a more potent greenhouse gas than CO_2 [4-7]). Inefficiencies in N uptake lead to significant N losses to the environment (on average over 50 kg N ha^{-1} year^{-1} [8]), causing eutrophication of aquatic ecosystems [9], and lowering groundwater quality [10]. Plant species richness and diversity has also decreased in many ecosystems [11,12], with many of these issues likely to be compounded by global climate change [13].

World demand for N fertilizer is projected to grow annually at 1.5% from 2015-2020, reaching 118.7 Tg yr^{-1} in 2020 [14]. This is driven by a population growth reaching 9 billion in 2050 and a global shift towards a more protein rich diet in developing countries [15,16]. In China, particularly after the Chinese Economic reform (i.e. 'reform and opening-up'), the average supply of animal-derived protein rose from 3.2 g capita^{-1} day^{-1} in 1980 to 39.3g capita^{-1} day^{-1} in 2013 (FAOSTAT, 2015). While veganism is rising in Western nations, consumptions of animal-derived protein remains high at 57 g capita^{-1} day^{-1} in Europe and 69 g capita^{-1} day^{-1} in the USA (measured from 2011-2013, FAOSTAT, 2017). Changes in diet are leading to rising N fertilizer demand under current practices, and are likely to cause further environmental issues. Hotspots of agricultural N fertilizer application have shifted from the US and western Europe in the 1960s to eastern Asia in the early 21st century [1]. Together Europe, China, and India now account for over 50% of the N fertilizer consumption globally (FAOSTAT, 2015).

Whilst global food production must increase globally, the land area dedicated for food production cannot expand further and may decrease to enable large-scale deployment of negative CO_2 emission technologies [17], limiting agricultural GHG emissions, increasing soil carbon sequestration [18,19], and maintaining soil health [20]. Now more than ever, N application for crop production must take into account the environmental consequences of the practices and possibly help to mitigate climate change.

Similar to the first Green Revolution in the 1960s and 1970s, progress will likely emerge from a combination of advances in the genetic stock of crop varieties accompanied by changes in agronomic practices and fertilizer products. The stakes are high and all aspects of science, from fundamental plant biology to agronomy, must work in an integrated manner to achieve sufficient food production with limited impact on environmental conditions [21-23].

Here we propose a roadmap to developing crop varieties with low N requirement. This builds on our understanding of N demand in contrasting environments, in major crop producing areas of the world (detailed in an initial section). We propose a shift in approach from quantifying the processes leading to high nitrogen use efficiency (NUE, See Glossary) to considering crop N requirement leading to optimal N application, and propose that N responsiveness is a useful trait that can be assessed and selected on by studying crop varieties under a range of N levels.

Worldwide crop production is dominated by four crops (FAO Stats 2017) sugarcane (*Saccharum officinarum*, 1,842 Mt. year^{-1}), maize (*Zea mays*, 1,135 Mt. year^{-1}), wheat (*Triticum aestivum* L., 772 Mt. year^{-1}) and rice (*Oryza sativa*, 770 Mt. year^{-1}). Interestingly, some sugarcane varieties are able to obtain up to 60% of their N through interactions with endophytic diazotrophs [24,25]. Efforts to exploit plant-microbes association to replace N fertilizer application have a high potential to reduce our reliance on synthetic N fertilizer, and are beyond the scope of this review [26-29]. We believe that our approach is complementary to those efforts, and that our roadmap in identifying low N requirement crops should also help identify genotypes that are amenable to better interactions with beneficial microbes. Wheat, maize and rice require high levels of inorganic N to be available in order to achieve high yields and many efforts have been put in place to improve NUE for these crops. Here we focus on wheat production as it has benefited from the Green Revolution with a trebling of global yields (1.1 $t.ha^{-1}$ in 1961 to 3.4 $t.ha^{-1}$ in 2016, FAOSTAT, 2018). Wheat is used as an example of a crop requiring high N levels both for yield and grain quality production [30], and we discuss similarities and discrepancies with maize and rice. The application of these principles to indeterminate flowering crops (such as pulses, potatoes, and brassica species) is beyond the scope of this review.

Understanding N demand in contrasting cropping systems: wheat production in Europe, India and China

Western Europe, India and China now account for 63.4% of worldwide wheat production (Fig. 1). In Western and Northern Europe, most of the wheat produced originates from winter varieties (autumn-sown and harvested in the summer, Fig. 1), which require a period of vernalisation (See Glossary) and are high-yielding compared to spring varieties. By contrast, in India wheat is grown over a short period of five months during winter (November-April), followed by rice, maize or cotton cultivation. Over 75% of national wheat production occur in the northwestern states of India, and as an example, in the Punjab region of northern India (representing 17.7% of the total Indian wheat production), fields are generally irrigated, which also dictates the timing of fertilizer application (Fig. 1). In China, winter wheat also represents the main part of crop production, and as in India, farmers predominantly use a wheat-maize double cropping system [31].

In the UK, the Nutrient Management Guide (RB209, AHDB) provides recommended fertilizer applications necessary to achieve high yields and a specific grain quality. Recommended N applications vary depending on soil type and N content, which is heavily affected by previous crops. Depending on the amount of N required, fertilizer can be applied in the form of ammonium nitrate, urea, or urea-ammonium nitrate liquid, as several dressings generally around the stem extension stage. Wheat produced for milling requires an additional late N application during development to increase grain protein content (GPC, See Glossary). Farmers frequently add N beyond the economic return because estimating N demand is difficult [32]. Here we also develop the concept of an “economic N optimum”, representing the point at which a financial penalty is imposed by the marginal gain in yield, relative to the additional cost of fertilizer.

In India, the blanket fertilizer N recommendations for irrigated wheat means that farmers apply fixed doses at specific stages, generally early on in the season (Fig. 1) without adjustment for spatial and temporal variability in soil N supply, which leads to a low percentage (30-50%) of applied N fertilizer being used by the crop [33]. Furthermore, N fertilizer is commonly applied in excess to avoid N deficiency and low yield, which is financially feasible since N fertilizer is heavily subsidised. Urea is the major form of N fertilizer representing about 83 % of the total fertilizer N production (FAI, 2015). In China, subsidised fertilizers also tend to be applied in excess (generally > 200 kg $N.ha^{-1}$ and ranging from 0 to 615 kg $N.ha^{-1}$ [34]), with potential yield inhibition in some instances [35]. In both China and India, recent initiatives in training farmers to monitor the crop N requirement have led to reduction in N application [35], Box 1 and Box 2). In India and

China, the size of holdings tend to be smaller than in Europe [36], which means that more farmers must be trained and likely have fewer bespoke decision support tools available to them.

Commercial wheat varieties are currently selected and marketed primarily for high yield. Additional traits include disease resistance, lodging, grain quality and GPC. However, high GPC (11-13% being necessary for bread produced through the Chorleywood bread process favored in the UK) is not a relevant criterion in India and China. The focus on high yield has led to the selection of wheat varieties with lower wheat grain quality in China because of the well-documented trade-off between yield and quality [30]. The evaluation of commercial varieties tends to be conducted under optimal agronomic conditions, including high N availability. Interestingly, there is little information defining the specific N requirement of each variety in many European countries (e.g. AHDB, Recommended List). By contrast some countries have adopted a different policy: in France, pre-registration varieties are tested under three N levels (optimal N level, deficient N level and over-fertilization) and their GPD (grain protein deviation, See Glossary) is also published (Section Céréales à paille du Comité Technique Permanent de la Sélection). The approach of the Danish government (detailed in Box 2) provides evidence that specific state-wide regulation can lead to the selection of varieties with a lower N requirement.

Lowering global N application relies on the cumulative decisions of millions of farmers as to whether or not apply N, by how much and when in the cropping cycle. These are complex decisions driven by many factors including N fertilizer cost, grain price, crop N demand, and factor specific to each cropping systems (e.g. irrigation timing). Specific actions must be taken to support farmers in achieving high yield production while reducing N application, and these falls into two broad categories (each highlighted by a large curved arrow in Fig. 2). (1) Cultivated varieties must be able to perform to a high standard under low N conditions. To this end, the selection of low N requirement crops will emerge from developing an efficient phenotypic selection process under low N conditions, as well as genetic markers for low N requirement, and overall establish low N requirement as a breeding target on par with yield and traits related to disease resistance or grain quality. (2) Simultaneously, variety-specific agronomic information and training in assessing crop N requirement should be more widely available to farmers to reduce field N application while maintaining high yield and quality. While we have focused here on wheat production under different cropping system, a similar evaluation of the different cropping systems for other cereal crops such as maize or rice would be highly relevant.

Re-thinking crop N requirement from high NUE to low N optima

In order to quantify the genetic differences in the processes associated with N uptake, translocation, assimilation and remobilization, Moll *et al.* introduced the concept of agronomic nitrogen use efficiency (NUE) [37]. Defined as the ratio of grain produced to the amount of N available to the plant (though other definitions have also been used [38]), it is a descriptive measure easily calculated from common measurements. NUE, though representing a complex trait, is a concept easily grasped and which is scalable i.e. measured at the plant, field and the global level [39]. However, this concept has many limitations. It is a ratio (expressed as % or as kg dry matter per kg N) that is meaningless in both commercial and environmental terms, it does not allow for easy comparison or for setting targets for improvement. It is rarely used and measured by farmers and breeders, and can only be calculated at the end of the growing season which prohibits an in-season change in N management practice [40]. Given that varieties grown under the same N level and showing higher yield, by definition also show higher NUE, improving yield seems sufficient to improve NUE. However, NUE is highly dependent on changes in environmental conditions [41] and tends to be negatively correlated with N availability [42]. So, a high NUE is achieved under conditions of low N availability, and a low NUE is achieved under conditions of high N availability. A facile means to increase NUE is via lower N inputs, but at some point this will lead to an unacceptable reduction in yield (Fig. 2).

The economic N optimum is defined as the N level necessary to achieve a high yield with the lowest input cost, so as to maximize profits [43]. Following this, applying N beyond the economic N optimum will result in a loss of profit for the farmer, while application below the economic N optimum will result in a yield loss (and corresponding loss of profit). Thus, the aim is to define agronomic conditions or develop varieties under which the N optimum is low whilst the yield is high (inset Fig. 2). Typically the economic N optimum, as shown in Fig. 2, is calculated from a N dose-response curve, which varies across varieties and fields [43]. The economic N optimum represents a meaningful measure of N supplied to the field and it could potentially be included in the information associated with commercial varieties, as done with the level of resistance to specific pathogens. It could also be adjusted to consider the environmental cost of N application, and could then work across different disciplines to become a broader and more integrated concept. In addition, considering the economic N optimum forces a consideration of the N requirement throughout the growing season. This would be useful for farmers, breeders, and scientists as discussed in the section above.

Defining a low N requirement ideotype linking to N responsiveness

The definition of economic N optimum is perhaps not seemingly as descriptive as that of NUE and a more difficult concept to grasp. In addition, the economic element makes it more difficult for scientists to include when conducting small scale experiments. N optimum is a trait that is not measurable at early developmental stages, and is more difficult to measure at small scale. Thus far, the focus on improving NUE and its components has provided a framework to understand the processes (e.g. N Utilization Efficiency, NUtE, and N Uptake Efficiency, NUpE) and over the last 50 years, our understanding of the principles and the key molecular players in N uptake, assimilation and utilization has grown significantly [44-47]. However, improving the efficiency of these processes has proven difficult. One clear advantage for considering the N dose-response curve is the shift in perspective that this offers (Fig 2). In essence, a variety showing a low N optimum is a variety that is highly responsive to N at low doses, and that continues to be responsive to external N even under N replete conditions. Thus, a high N responsiveness that is maintained under high N conditions becomes a desirable trait along with the traits already extensively defined for selecting a low N economic requirement crop [40,42,48].

We define N responsiveness in the context of external N availability and uptake, but this can be affected by the plant's internal (intrinsic) N level or N status (See Glossary). Split-root experiments (See Glossary) have been highly useful in demonstrating how plants integrate responses to external N availability and N status, which are genetically distinct (e.g. [49]). These experiments also provided some information on the signaling components associated with monitoring internal N status and response to soil N availability [49,50]. The selection for high N responsiveness has already happened to a certain level since modern varieties are more responsive to external N availability compared to older varieties or landraces [51,52]. However, to achieve high N economic optimum, high N responsiveness must be maintained even under N replete conditions, i.e. when the intrinsic N status of the plant is high. This is a challenging target for breeders.

Establishing the molecular basis for high N responsiveness

Focusing on N responsiveness also provides a link to some of the most exciting questions and developments in the field of N research: (1) how is environmental N sensed and the signal transduced into a phenotypic response? (2) how is plant N status monitored? and (3) how is plant development and primary metabolism regulated depending on external N availability and internal N status? To achieve a low N optimum, physiological and biochemical processes need to be efficient in plants grown under high as well as under low N conditions.

Significant progress has been made in our understanding of environmental N sensing and signaling in recent years. These have been summarized in many reviews [53-55]. Specific components of the N sensing apparatus have been identified in the model species *Arabidopsis thaliana* such as the NRT1.1 nitrate transceptor (i.e. a protein facilitating nitrate transport across the plasma membrane that also holds a role in signaling [56]), components of the signaling cascade downstream of the Ca^{2+} secondary messenger [57, 58] including the kinases CIPK8 [59] and CIPK23 [60], or transcription factors (TF) such as ANR1 [61], NLP7 [62] or SPL9 [63]. Though much work remains to be done to characterize orthologs in rice, maize or wheat, those identified thus far tend to have conserved function [64-67].

Many elements involved in how plants monitor their N status have been proposed, a clear mechanism has yet to be fully established [53,68]. In this context root-shoot-root signaling is paramount and small peptides have been implicated. For example, CEP acts as a root derived ascending N-demand signal to the shoot where it is perceived by CEPR which leads to a putative shoot-derived descending signal that up-regulates nitrate transporters in the roots [69]. The existence of at least two genetically independent systemic signaling mechanisms reporting the N supply and demand of a plant have been reported [49], also placing cytokinins as crucial component of a root-shoot-root signaling/relay mechanism [70]. In addition, GARP TFs have also been implicated in the N starvation response [71]. With regards to the elements regulating the N response dependent on N status, much remains to be done in wheat.

Understanding the regulation of primary metabolism and studying the physiology, biochemistry and molecular processes under low vs. high N conditions should provide information on how these processes are regulated depending on N status to achieve low

N optimum. A systems biology approach may be useful and has already led to identifying a role for CCA1, a master circadian clock regulator, in regulating N-assimilatory pathways in *Arabidopsis* [72,73] and the BT1/BT2 TF that repress high-affinity nitrate transporters expression, leading to overall low NUE in both *Arabidopsis* and rice, under low N conditions [74]. While components of central metabolism are heavily regulated at many levels, there are successful examples of upregulation in wheat such as the over-expression of the chloroplastic isoform of glutamine synthetase (a key enzyme in assimilating ammonia in organic compounds) that lead to increased grain yield and spike number under both high and low N conditions [75]. Ultimately, transcription factors may provide a useful route to modifying primary metabolism, such as the Dof1 TF that when over-expressed in *Arabidopsis* lead to greater yield production under low conditions [76], and greater yield in wheat [77].

Work in *Arabidopsis* has been critical in identifying key elements of plant N response. The abundance of resources available and the agility and speed with which experiments and hypotheses can be tested in *Arabidopsis* must be exploited to achieve low N requirement in crop. However, rather than simply assuming that a response measured in *Arabidopsis* will translate in a crop, these must be re-tested in the species of interest. Translating useful information on N metabolism and regulation from fundamental research in model species about N metabolism and regulation to practical advances in crop N requirements necessitates numerous technical advances. These are now becoming available in many crops. In wheat, for example, these include (1) the availability of a near complete genome sequence with improved annotation [78-81], (2) the high efficiency for genetic modification through the Agrobacterium method [82,83], the availability of CRISPR/Cas9 [84], (3) TILLING lines [85], (4) the possibility of generating hybrid wheat [86] and (5) rapid generations via speed breeding [87]. Many of these advances have already been achieved in rice and maize, allowing researchers to rapidly investigate specific mechanisms directly in crop species.

Efficient screening for low N requirement varieties and assessment leading to tailored agronomic recommendations

NUE is a complex trait controlled by many genes [88,89], and it is highly likely that genetic studies for high N responsiveness will also identify many underlying loci. Genetics studies in French and Australian wheat varieties have shown that considering the response to increased N can highlight previously unidentified genetic regions of interest [90-94]. This complex genetic basis will make classical marker assisted selection (MAS) difficult but may be more amenable to genomic selection to select for cumulative additive variation for N response [95]. Landraces and pre-breeding material also offer a novel avenue for exploitation of natural variation with regards to N responsiveness.

Economic N optimum itself cannot be used as a definitive breeding target. However, high yield under low N conditions together with N responsiveness measured as the yield difference between low and high N conditions is quantifiable and tractable and would provide sufficient description for a selectable N requirement breeding trait. Thus, we support the idea that varieties should be selected and assessed under low N conditions as well as optimal N conditions [93], and that variety-specific N requirement information and agronomic recommendations to achieve high yield should be made available to growers and farmers through documentation akin to the Recommended List in the UK (Fig. 2). An argument put forward against selecting varieties under low N, is the low level of homogeneity and low heritability of yield under low N vs high N conditions. However, Hitz et al. [96] showed that without breeding lines under low N it is not possible to identify low N requirement genotypes.

Technological advances mean that field phenotyping has rapidly improved both in terms of capacity and accuracy, due to recent technological advances [97,98]. Screening varieties for low N requirement, especially N responsiveness, present an added difficulty in the establishment of growth conditions at different N levels. To address this, protocols have been developed for opti-plots trials that enable the testing of many varieties under multiple N levels across a single plot within a single field [99]. The smaller scale of these experiments enables commercial varieties as well as pre-breeding material, for which seed supplies are limited, to be tested for N response in relation to yield. One outstanding question is whether this system could be scaled down even further to allow for accurate selection (on both yield and N responsiveness) at an even earlier stage in a breeding program providing a predictive tool for estimate N response.

Thus far, no simple physiological marker has been identified that is easily measurable and could be integrated in breeding programs, akin to the relative abundance of ^{13}C ($\Delta^{13}C$) for the selection of drought tolerant wheat cultivars. In this case, water use efficiency (WUE) was shown to be negatively correlated with $\Delta^{13}C$ in wheat dry matter [100]. While selecting varieties showing a high WUE by making physiological measurements of WUE would be too time consuming and prone to errors, $\Delta^{13}C$ analyses are much more feasible and could be integrated in breeding programs which ultimately lead to the selection of drought-tolerant commercial varieties [101]. Conversely to the link between $\Delta^{13}C$ and WUE, the natural abundance of ^{15}N has not been consistently established as proxy for low N requirement, and its link to the efficiency of specific physiological processes is less well established [102]. Thus far the abundance of ^{15}N has mostly been used as a tool to study the N partitioning rather than an indicator for N responsiveness [103,104]. However, the concentration and activity of the primary carboxylase, RuBisCO is a major N sink, and also affects $\Delta^{13}C$ depending on the carboxylation strength and drawdown of internal CO_2 within the leaf. The relationship between N content and $\Delta^{13}C$ is one avenue of research which provides a promising marker for low N requirement.

Concluding Remarks and Future Perspectives

Decreasing crop N requirement while maintaining high yield is necessary for sustainable future production of wheat and other cereals. It is also important to curb pollution both due to N fertilizer production and N leakage to the environment. Although game-changing projects aiming to develop cereals with the capacity to fix N_2 through the establishment of symbiotic relationships with diazotrophic bacteria are underway [105], they remain in their infancy. Thus, shorter term solutions are necessary to reduce crop N requirements and improve efficiency of N fertilizer applications, and may also lead to further mitigation of GHG production [18]. Considering N responsiveness and its underlying mechanistic regulation, as well as introducing varietal or advance breeding line screening varieties for low N requirement and high responsiveness varieties within crop breeding pipelines is likely to provide the new means to develop new varieties with a low economic N optimum. Our roadmap provides a useful translational framework for researchers, breeders, agronomists and farmers to work together in achieving low N requirement crop worldwide (see also outstanding questions).

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Box 1: PAU-Leaf Colour Chart technology: A breakthrough research for defining low N optimum for different field crops

Precision N management techniques generally require expensive equipment (e.g. optical sensors, chlorophyll meters and plant N analysis techniques) and expertise to assess crop N status. The Punjab Agricultural University (PAU) has developed a Leaf Colour Chart (LCC) (see Figure I) as a useful low-cost tool to support decision making on the timing and quantity of N fertilizer application. They have adapted an initial concept of using leaf greenness to inform fertilizer N application timings, developed for rice management by the International Rice Research Institute (IRRI, [106]). The PAU-LCC has been adapted for multiple crops, such as rice [107,108], maize [108,109], wheat [110,111], direct seeded rice, basmati rice and cotton [112].

The PAU-LCC consists of a series of graded panels with differing shades of green coloration, that is used to compare with the colour of the adaxial leaf surface (Fig Box1). It is low-cost (£1) and easy to use, farmers can be easily trained to assess the N requirement of their crop in a day. The colour of the first fully exposed top leaf of randomly selected plants is assessed and the assessment can be conducted at specific growth stages (e.g. from 14 days after transplanting to initiation of flowering in rice, from 21 days after planting to initiation of silking in maize, at Zadoks growth stage 29 in wheat and at thinning and initiation of flowering in cotton). The PAU-LCC also provides information regarding the required fertilizer N doses.

The dissemination of this low-cost tool to a model village (Bassian, Ludhiana, Punjab) has led to a reduction in N application of an average 75 kg N.ha^{-1} in rice (2017) and 50 kg N.ha^{-1} in wheat (2017-18).

Box 2: *Country-wide policy-driven restrictions in N applications can be effective in driving reduction in N use*

China has implemented a series of policies related to N fertilizer to promote grain production thus ensuring food security. These policies cover nearly all aspects of agricultural production including subsidies for N fertilizer production (e.g. discount of energy consumption, transportation costs and taxes), direct payments for grain producers, comprehensive subsidy on agricultural inputs, seed variety subsidy, subsidy for purchase of agricultural machinery, and the complete cancellation of agricultural taxes [113,114]. However, Chinese grain production is dominated by millions of smallholder farmers who apply the concept of “more fertilizer, higher yield”, which makes difficult to improve N management technology and large-scale production [35]. In recent years, China’s agricultural policy has gradually begun shifting towards sustainable development. In addition to vigorously promoting organic fertilizers (mainly based on organic fertilizers subsidies), the Ministry of Agriculture of the People's Republic of China has also issued the “Zero Growth of Chemical Fertilizer and Pesticide Use by 2020” to vigorously limit chemical fertilizer use and improve efficiency [115].

China's N fertilizer application gradually stabilized from the early 2010s. This progress was mainly driven by the extensively investigation of the farmland N loss pathways and the relevant control measures [116,117], as well as the improved field N management practices [118]. A comprehensive decision-support integrated soil-crop system management program was implemented in 452 counties with a total of 37.7 million cumulative hectares in the past decade. This program successfully improved 10.8% of wheat yield with 18.1% of N reduction [35]. This shows that reducing N application, thus increasing sustainable production is compatible with continuous growth and food production.

Denmark, for which 60% of land is used for agriculture, has significantly reduced the N upload in the environment through a series of effective policies action plans, since the mid-1980s [119]. Total agricultural N input has decreased from 662 Gg N in 1983 to 448 Gg N in 2012 [119]. A main driver for this was a 50% reduction in application of synthetic N fertilizer, which peaked in 1989 at 189 kg N ha-1. They have shown an increased in N efficiency for the agricultural sector from 20-30% to 40-45%. The Netherlands also reduced the N fertilizer application in response to European environmental policies and regulation, while yield doubled [8]. Overall, these initiatives suggest that applying specific restrictions can drive some innovations, leading to a decrease in N requirement while maintaining the yield.

Figure legends:

Fig. 1. Schematic of cropping systems in Western Europe, India and China.
Wheat production in Western Europe, India and China represent a large proportion of the worldwide production ([1] In bracket Percentage of global wheat production) and cropping systems varies amongst regions. Here a typical cropping system is detailed for each region and shows the time of wheat planting in the field, N application and irrigation, as well as harvest. For example, in Europe, the first N application occur at Zadoks Growth Stage (GS) 23, which correspond to the appearance of tillers, while the second and third N application occur around GS31 (i.e. stem elongation).

Fig. 2. Framework for producing low N requirement crops. The economic N optimum provides a framework facilitating exchange of information amongst disciplines and leading to new lines of enquiry to produce high quality grain under sustainable conditions. The economic N optimum is calculated from the yield response curve under increasing N levels and is defined as the N level necessary to achieve high yield with the lowest input cost while maximizing profits. Producing crops under sustainable conditions will emerge from collaborative work amongst geneticists and breeders, agronomists and plant scientists that will be facilitated through our proposed roadmap. Targeted questions with specific relevance to the selection or cultivation of N-efficient wheat can inform research programmes. Knowledge acquired from both translational and fundamental research can inform variety selection and agronomic practices. Characteristics of the crop ideotype with low economic N optimum falls into three categories: (1) High grain number per ear, larger grain with required quality, (2) efficient photosynthesis, carbon and N partitioning traits leading to high N responsiveness, balanced number of tillers, limited stem extension, suitable for high density planting, low NH_3 emission, and (3) extensive root system for efficient nutrient capture at different depths throughout the plant life cycle, and amenable to interactions with beneficial soil microorganisms.

Figure I. Box 1. The PAU-leaf color chart is used to assess the N demand of wheat plant, by comparing the color of the leaf to the gradient of greenness on the card. Specific advices are provided on the back of the card so the farmers can decide to provide or not additional fertilizer.

Glossary

Economic N Optimum, N fertilizer rate beyond which a financial penalty is imposed by the marginal gain in yield, relative to the additional cost of fertilizer

GPC, grain protein content, high GPC is associated with high grain quality

GPD, grain protein deviation, a positive GPD is desirable as this indicates a higher GPC considering the yield (GPC and yield are generally negatively correlated under a constant N supply).

NUE, ratio of grain produced to the amount of N available to the plant

N responsiveness, corresponds to the capacity of plants to induce morphological and physiological changes to N external availability in order to induce N uptake and assimilation

N status, whether a plant is overall N-replete or N-deplete

Opti-plot trials, field trial in which each variety is grown under at least four and often six N level, in order to calculate the economic N optimum from yield data

QTL, quantitative trait loci analysis, association of specific phenotypic traits to genetic markers

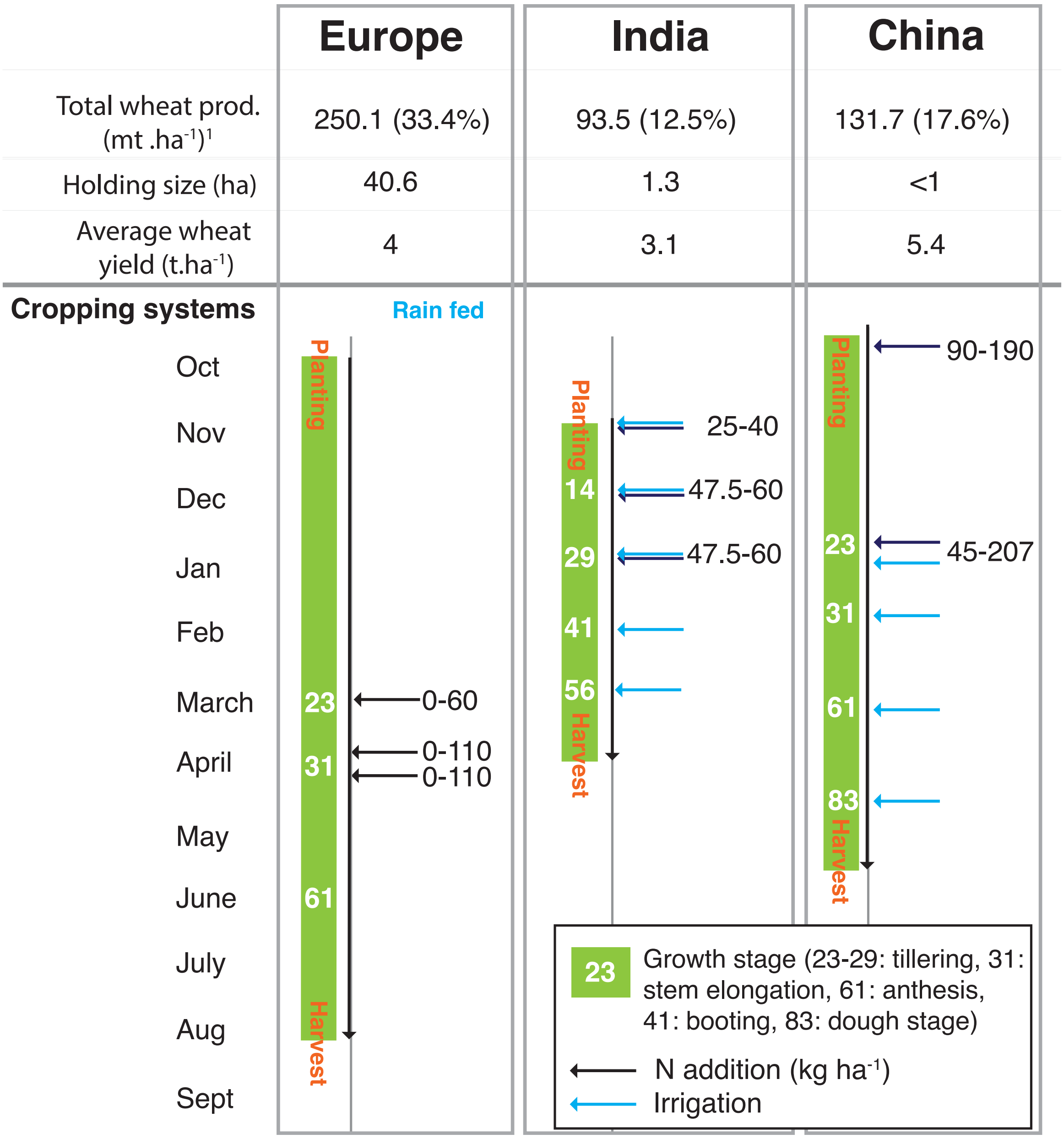
Split-root experiments, experiments where the root system is separated in two sections one section exposed to N while the rest of the root system is starved

Vernalisation, the programmed physiological process in which prolonged cold-exposure provides competency to flower in plants; it is necessary for winter wheat varieties to reach the reproductive developmental stage.

OUSTANDING QUESTIONS

- Can N responsiveness be a good indicative marker for low N optimum?
- What is the genetic basis for N responsiveness?
- Are elements of the N status monitoring apparatus conserved amongst plant species?
- How is plant primary metabolism regulated under low vs high N status?
- How can we better estimate N availability?
- How can the plant influence soil microorganisms to increase N availability?

Figure 1



	Europe	India	China
Total wheat prod. (mt .ha^{-1})^{1}	250.1 (33.4%)	93.5 (12.5%)	131.7 (17.6%)
Holding size (ha)	40.6	1.3	<1
Average wheat yield (t.ha^{-1})	4	3.1	5.4

Fig. 1. Schematic of cropping systems in Western Europe, India and China.

Figure 2

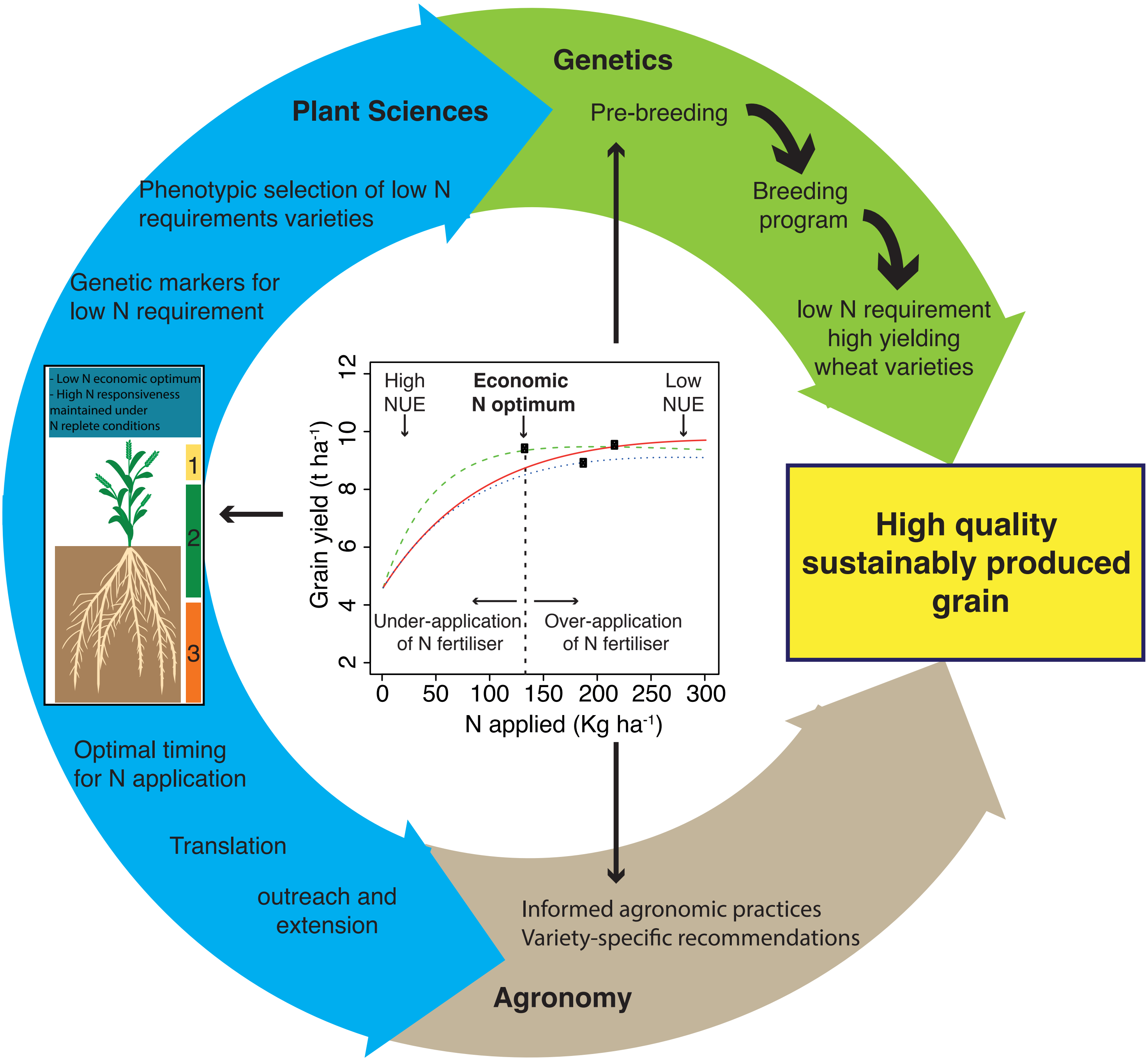


Fig. 2. Framework for selecting crop with low N requirement.

Figure I, Box 1

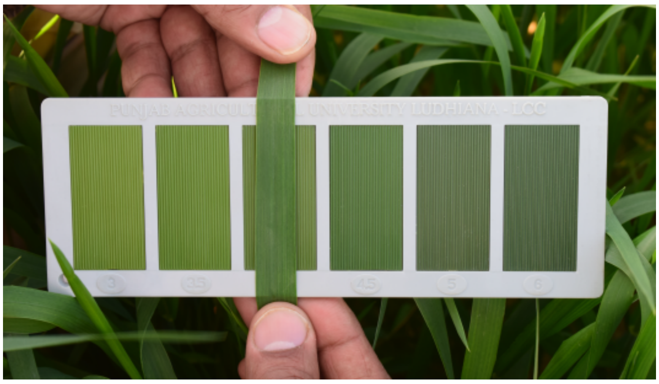


Fig. Box 1- PAU-Leaf Color Chart (LCC) to monitor crop N demand