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Water and nitrogen use efficiency in plants and crops

Routes to reducing the N requirements of high yielding wheat crops

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Summary

Improving the efficiency of nitrogen (N) use to meet the aims of growers, end-users and society requires that grain yields are maintained or increased whilst N requirements of crops are reduced. The N use efficiency (NUE) concept does not adequately capture these aims. We therefore adopt the term HYLO – High Yield, Low N Optima. Reducing grain protein content of varieties seems one of the most promising routes to reducing N requirements of feed wheat. However, improvements in recovery of N from soil and fertiliser, as well as improvements to N harvest index, are the only routes currently available for bread wheats. Development and commercialisation of HYLO varieties will depend crucially on there being appropriate measures in variety testing systems to distinguish low N requirements.

Key words: Nitrogen fertiliser, protein, NUE, wheat, variety testing

Introduction

Fertiliser nitrogen (N) is of major economic importance in growing wheat crops, commonly doubling yields. This increase in yield is very important in preventing expansion in the arable area and associated negative impacts on the environment, as well as meeting the food security demands of a growing population (Snyder *et al*., 2009). However, the use of N fertiliser can be responsible for over 75% of the GHG emissions from crop production (Kindred *et al*., 2008) and it is also a significant source of water and air pollution. Consequently, improving crop yields without increasing (and ideally by reducing) N fertiliser use is highly desirable.

N fertiliser use can be reduced, or yield increased at a given N application rate, either by improved N management or by crop genetic improvement. Good N management principally involves anticipating optimal N rates and applying them at optimal timings, taking into account crop growth, weather and soil conditions. It has been estimated recently that English agricultural GHG emissions could only be cut by up to 2.1% through improvements in N management to wheat, barley and oilseed rape, whereas genetic improvement could reduce emissions by up to 5.0% (Berry *et al*., 2010). This paper explores how we might best achieve this genetic improvement to give wheat crops with high yields and low N requirements.

Defining N requirements

The N requirement of a UK crop is generally defined as the rate of fertiliser N that should be applied to maximise profitability for the grower; these are predicted by UK recommendation systems (e.g. RB209, Defra, 2010; HGCA Wheat N management guidelines, Sylvester-Bradley, 2009). The N requirement or optimal N rate is the rate at which any further increase in N rate will cost more in N fertiliser than the value of the extra grain produced. This is therefore dependent on the relative price of grain and N fertiliser, or the breakeven ratio (BER): the amount of grain (kg) required to pay for one kg of N fertiliser. The BER is currently around five but has varied between three and nine over recent years. Yield responses to N diminish through the full range of N amounts, and the N optimum occurs at the point in the N response curve where the yield response slope is equal to the BER. In general, over the range 3–9, an increase in the BER of 1 reduces the N optimum by around 10 kg ha^{-1}. The N optimum can be determined from an N response experiment with a bare minimum of four N rates, but ideally with six or more N rates. This allows a curve to be fitted from which slopes and N optima are determined (see Sylvester-Bradley *et al*., 2008). A 'linear plus exponential' (LPE) function has been adopted for curve fitting in the UK through the last 25 years (George, 1984):

$$y = a + b.r^{N} + c.\mathrm{N}$$

where y is yield in t ha^{-1} at 85% DM, N is total fertiliser N applied in kg ha^{-1}. a, b, c and r are parameters determined by statistical fitting that tend to be associated with features of the response as follows:

a: the asymptote, or maximum achievable yield.
b: the change in yield from the maximum if no fertiliser N was applied.
c: the slope of the response beyond the region of maximum curvature. Where large N rates cause increasing yield loss (e.g. due to lodging), c tends to be increasingly negative.
r: the shape of the response in the region of maximum curvature. This value tends to be larger for flatter response shapes and smaller for sharper response shapes (i.e. those with a more distinct shoulder).

Optimum N rates are derived from the fitted LEXP parameters as follows:

$$\mathrm{Nopt} = [\ln(k\text{-}c)\text{-}\ln(b(\ln(r)))]/\ln(r)$$

where k is the BER (tonnes kg^{-1}) between fertiliser N (p kg^{-1}) and grain (p tonne^{-1}).

Due to the flatness of the response around the optimum, its determination is generally imprecise; i.e. there is only a small change in slope over a wide range in N rates. When comparing genetic differences it is rarely possible to do so at six N rates, so most studies investigating genetic improvement of N use efficiency have used only one or two N rates, but occasionally up to four (e.g. Barraclough *et al*., 2010).

The N requirement for a given variety within a given environment is commonly explained as being a consequence of the demand for N from the crop (crop N demand), the supply of N available from the soil (soil N supply) and the recovery of applied fertiliser N (fertiliser N recovery) as follows:

$$\text{N Fertiliser Requirement (kg ha}^{-1}\text{)} = \frac{\text{Crop N demand (kg/ha) – Soil N Supply (kg/ha)}}{\text{Fertiliser N recovery (\%)}}$$

Differences in soil N supply generally constitute the largest component of the environmental variation in N requirement; where soil N supplies are large N optima can commonly be below 100 kg ha^{-1}, where they are low N optima are commonly greater than 250 kg ha^{-1}. Environmental variation in crop N demand through effects on yields and grain protein can also affect N optima. Some yield effects may be offset by compensatory changes in N recovery or grain protein:

- reduced yields due to delayed sowing are offset by poorer N recovery (Webb *et al*., 1995),
- higher yields can be associated with higher N recovery (Bloom 1987)
- increased yields after break crops are associated with reduced grain protein (Vaidyanathan *et al*., 1987).

However, low yields on light soils are associated with increased N recovery (~70%; Webb *et al*., 1998) and low yields on chalky soils are associated with poor N recovery (~55%; Grylls *et al*., 1997).

Considering gross genetic effects, variation in all three components of the N requirement occurs, but differences in crop N demand are normally more important than differences in soil or fertiliser N recovery. For instance, breadmaking varieties generally yield less than feed varieties but their N requirements have been found to be greater (when fertilised optimally for yield, ignoring differences in grain value) because grain protein of bread varieties averages at 12% (2.1% N in DM) compared to feed varieties with about 11% protein (1.9% N in DM); a comparison of N response experiments since the 1980s shows the N optima of breadmaking varieties to be around 20 kg ha^{-1} greater than feed varieties (Sylvester-Bradley & Clarke, 2009). Breadmaking varieties also often require additional late N to achieve target grain protein of 13%; to increase grain protein by 1% generally requires around 50 kg ha^{-1} additional N (Sylvester-Bradley, 2009). The challenges for genetic reduction of the N requirements of milling wheats are thus more challenging than for feed wheats.

Defining the target for genetic improvement

Use of fertiliser N must be reduced if GHG emissions from arable agriculture are to reduce in line with targets accepted by the UK Government. However, unless consumption of grain is similarly reduced, any reduction in N fertiliser use should not be at the expense of yields, because this will simply 'export the problem', increasing land use change to arable production elsewhere in the world. If consequential land use change occurs on high carbon stock land (e.g. forest or grassland) then the CO_2 emissions from depletion of soil carbon can seriously outweigh the savings in emissions from reduced N fertiliser use (Kindred *et al*., 2008). It is therefore clear that the aim should be to reduce N fertiliser requirements whilst maintaining or increasing grain yields, or at the least, maintaining N fertiliser requirements whilst increasing grain yields. Such varieties may be described as High Yield, Low Optima (HYLO). For milling varieties the target may be described as high yield & protein, low optima (HYPLO).

Defining nitrogen use efficiency

Much research has been undertaken with the aim of improving Nitrogen Use Efficiency (NUE) (see reviews by Foulkes *et al*., 1999). NUE is however often ill-defined (Brauer & Shelp, 2010). The most widely used definition, given by Moll *et al*. (1982) is the grain yield per unit of N available to the crop from soil and fertiliser. Other studies however define NUE as the proportion of available N captured in the harvested grain (e.g. Raun & Johnson, 1999), sometimes called grain N recovery (Jenkinson & Smith, 1988). For the majority of wheat production, N fertiliser is predominantly applied to increase grain yields because wheat is valued by weight at a fixed moisture content i.e. for its energy content rather than its protein content. Only a minority of UK wheat production is influenced by premia for high protein. So, when considering NUE in wheat primary importance should be attached to production of grain yield, rather than grain protein yield or grain N recovery; Moll's definition would thus seem to be the most appropriate.

NUE can be taken to have two components; N uptake efficiency (NUpE) and N utilisation efficiency (NUtE). NUpE is the proportion of N available to the crop from soil and fertiliser that is taken up by the crop, thus is equivalent to soil plus fertiliser N recovery. NUtE is the efficiency with which N in the crop is used to produce grain dry matter (kg grain DM per kg N in the crop). This encompasses a wide range of processes including partitioning of N in the crop and its use in forming green area (canopy N ratio), the effectiveness of this green area in intercepting light (extinction coefficient), the survival of the green area (senescence), the efficiency with which light is converted to dry matter (radiation use efficiency), and the partitioning of dry matter to the grain (harvest index) (Sylvester-Bradley & Kindred, 2009).

The measured NUE at a site varies greatly with the level of N supply, usually reducing as N supply increases, and the relative importance of NUpE and NUtE changes also (Sylvester-Bradley & Kindred, 2009). The N rates used in comparative studies can therefore have a major effect on the conclusions drawn. Also NUE rarely relates to the N requirement of the crop as would be applied on-farm. Thus Sylvester-Bradley & Kindred (2009) suggested that NUE should be expressed at the N optima (NUEopt) and that studies should attempt to determine NUEopt when seeking to improve N efficiency.

Evaluating measures of N efficiency – Relating NUE to yields and N optima

To achieve HYLO and HYPLO varieties we need effective measures that discriminate varieties that have high yields and low optima from others which may have high yields but high optima, or low optima but low yields. Fig. 1 shows N responses for a range of hypothetical variety types, produced by varying parameters of the LPE curve so that differences in yield and N optima can be compared with differences in calculated NUE at a range of N rates.

There are a number of possible improvements in the shape of the N response curve that might apply to HYLO type varieties compared to a standard variety.

- Maintained yield, low optima (MYLO)
    - Via higher nil-N yield (MYLOHY0) (less negative '*b*' parameter) and/or
    - Via steeper shoulder (MYLOMY0) (lower '*r*' parameter)
- higher yield, maintained optima (HYMO) (higher '*a*' parameter)

Previous work has shown a positive relationship between the yield potential of a variety and its N optimum (Sylvester-Bradley *et al.*, 2008), so we must also consider N responses of high yield, high optima (HYHO) and low yield, low optima (LYLO) varieties.

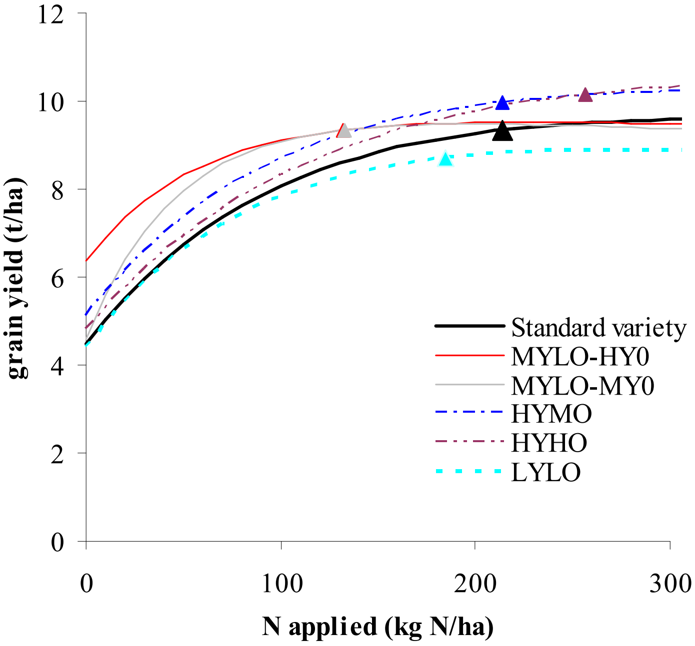

Fig.1. Example N responses and N optima for a range of variety types (see text for descriptions of each) fitted using the LPE function with parameters as in Table 1.

Table 1 shows the response parameters of theoretical HYLO varieties adjusted to give substantial reductions in N optima without effects on yields at the optima. There is very little difference in NUE measured at a fixed N rate (e.g. 200 kg ha^{-1}), and a difference in NUE at zero-N only occurs where the lower optimum is primarily a result of a high unfertilised yield, rather than a steep response to N (small *r*). A high NUE at nil-N can also result from a variety with high yield and high optimum. Whilst NUE measured at the optimum provides a fairer comparison, and shows the much improved efficiency of the HYLO varieties, it should be noted that it does not recognise a large benefit from varieties which give higher yields without increased N optima. NUE$_{opt}$ can also be high for varieties giving low yields and low optima, whereas these are unlikely to be

Table 1. *Effectiveness of N efficiency measures*

	Standard variety	MYLO-HY0	MYLO-MY0	HYMO	HYHO	LYLO
LpE parameters						
a	10.47	9.86	9.89	11.20	11.10	10.00
b	-6.0	-3.5	-5.3	-6.0	-6.3	-5.6
c	-0.0020	-0.0012	-0.0017	-0.0020	-0.0010	-0.0030
r	0.9900	0.9830	0.9789	0.9900	0.9915	0.9890
N optimum (kg ha^{-1})	214	132	132	214	257	185
Y @ N optimum (t ha^{-1})	9.34	9.34	9.34	10.07	10.14	8.72
NUE @ 0 kg ha^{-1} (kg kg^{-1})	47.5	67.6	48.8	55.3	51.0	46.8
NUE @ 100 kg ha^{-1} (kg kg^{-1})	38.1	43.0	42.9	41.6	39.3	37.1
NUE @ 200 kg ha^{-1} (kg kg^{-1})	28.1	28.9	28.8	30.3	29.6	26.7
NUE @ 300 kg ha^{-1} (kg kg^{-1})	21.4	21.2	21.0	22.8	23.1	19.9
NUE @ N opt (kg kg^{-1})	27.0	37.4	37.4	29.1	25.6	28.0
Margin over N cost (£ ha^{-1})	993	1041	1043	1080	1063	936
GHG emissions kg CO_2e t^{-1}	584	470	470	542	593	582
IRI-200	1.000	1.387	1.004	1.078	1.020	1.038
NPI-200	1.000	1.460	1.050	1.255	1.131	0.933

adopted commercially. Other indices using yields with and without N fertiliser can be derived on a similar basis to those used in water use efficiency studies (Ober *et al*., 2004); Infertility Resilience Index (IRI) and a Nitrogen Productivity Index (NPI) presented in Table 1 are calculated as follows, where ‘mean yields’ refer to averages across all the varieties in a site-season:

$$\text{IRI} = (\text{yield}_{\text{lowN}} / \text{yield}_{\text{highN}}) / (\text{mean yield}_{\text{lowN}} / \text{mean yield}_{\text{highN}})$$

$$\text{NPI} = (\text{yield}_{\text{lowN}} / \text{mean yield}_{\text{lowN}}) * (\text{yield}_{\text{highN}} / \text{mean yield}_{\text{highN}})$$

The IRI describes the proportion of high N yield maintained at low N, normalised by the average for the site season. The NPI multiplies the yield at low N, as a proportion of the site average, by the yield at high N, again as a proportion of the site average, thus ‘rewarding’ varieties which perform well at both high and low N. These indices may aid variety comparisons across sites and are simpler to use than NUE_{opt}. However, they can only reward low optimum varieties if a low optimum is associated with high nil-N yield, and high yielding varieties are rewarded even if their optima are high. In short, no metric from experiments with only 2 N rates conveys satisfactory information about yield and N optimum.

Evidence for Genetic Variation in N Requirement

Whilst a considerable amount of research has assessed genetic variation in NUE (e.g. Le Gouis *et al*., 1996; Barraclough *et al*., 2010), very little has sought to characterise genetic variation in N requirement. Sylvester-Bradley & Kindred (2009) reported N response experiments comparing old and new varieties in winter wheat and spring barley, and showed for wheat that N optima increased with increasing varietal yields (by around 20 kg N per t grain) but not in barley.

The data of Sylvester-Bradley *et al.* (2008) show little evidence of consistent differences in N response or N optima of individual modern varieties, though the experiments, with only two modern varieties in each trial, were not designed to examine variation between specific varieties. Effective tests of varietal variation in N optima should include a large number of N rates in a series of experiments, ideally across multiple environments.

Perhaps the best data available are from experiments conducted through the Wheat Genetic Improvement Network (WGIN; data available at www.wgin.org.uk/wgin_2003-2008/Resources/DiversityTrialGrainYields.xls) and reported by Barraclough *et al.* (2010). N optima for the 24 common varieties tested (Fig. 2) have not been reported previously. Given the uncertainties in fitting curves and N optima to only four N rates, there was some consistency in N optima across trials, some but not all of which was associated with differences in grain yield (Fig. 3). Whilst varieties with the low N optima tended to be low yielding (e.g. Mercia) varieties with lower grain protein also tended to have low N optima (e.g. Riband & Beaver) whilst high protein varieties tend to have high N optima (e.g. Hurley).

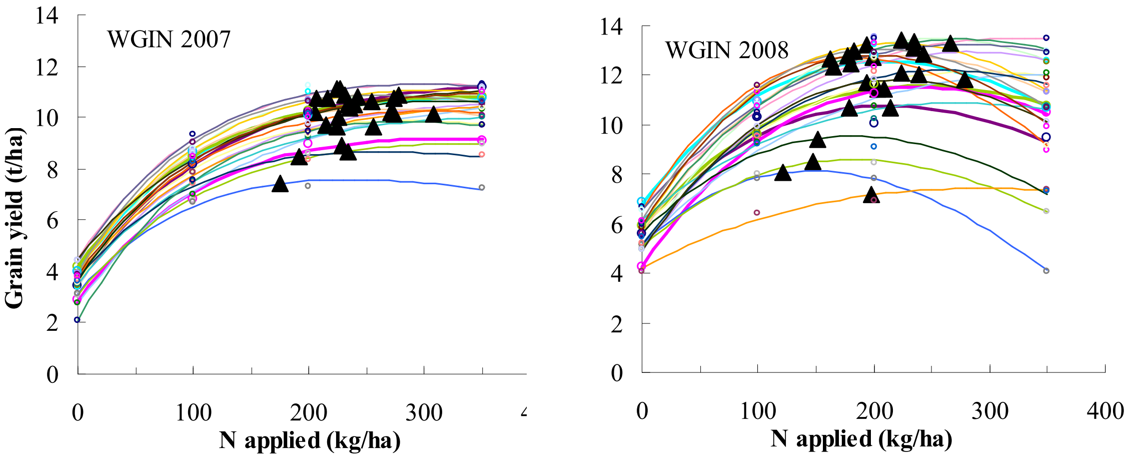

Fig. 2. N responses of varieties from the WGIN NUE Diversity experiments at Rothamsted (see Barraclough 2010, data by permission of M Hawkesford & P Barraclough; curves & optima fitted by ADAS).

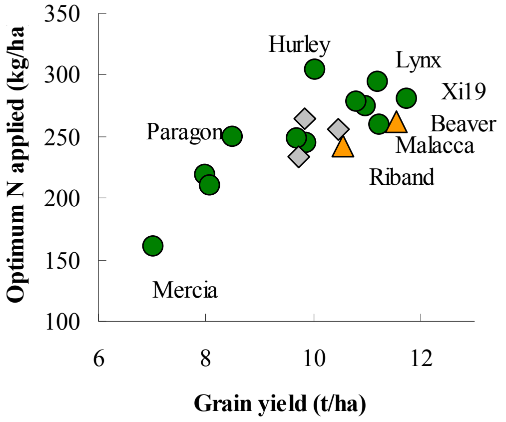

Fig. 3. N optima and optimum grain yields averaged from WGIN trials in 2007 & 2008 for breadmaking (circles), feed (triangles) and unadapted (diamonds) varieties.

Traits associated with low N requirements

Other than the WGIN Diversity trials, we are not aware of phenotyping studies made in multi-N-rate experiments where N requirements of varieties could be properly assessed; most N efficiency phenotyping has only been conducted at two N rates. Taking high nil-N yield as being indicative of low N requirement, data from the GREEN grain project (Sylvester-Bradley *et al.*, 2010) shows a negative association with grain protein and also straw N% and with N in the fertilised canopy at flowering, and a positive relationship with delayed senescence, N harvest index and delayed senescence (Sylvester-Bradley *et al*., 2010). However, heritability of canopy and N uptake traits was generally poor. Recent multi-species experiments have shown that triticale can outyield wheat by 20% with an N optima 20% lower, and that oats can achieve substantially higher yields than other species when N fertiliser is withheld (Kindred *et al*., 2010). An understanding of the traits causing these species differences could be useful in prioritising target traits for improvement in wheat.

Routes to HYLO & HYPLO wheat varieties

Crops need N to form green tissues that intercept light and form dry matter through photosynthesis, as well as to form protein in the grain. In modern wheat crops the requirement for N in the grain protein exceeds the theoretical requirement for N in the canopy to intercept sufficient light for current yields (Sylvester-Bradley *et al*., 2010). Reductions in grain protein demand can therefore be envisaged which could be neutral or positive for yield (especially if early translocation of N from senescing leaves to grain is reduced), with likely reductions in N requirement. The GREEN grain project set out an ideotype for wheat with a low N requirement and high energy content grain, achieving a reduction in N optimum of 40% without affecting grain yields. Associated traits were modest improvements in recovery of soil & fertiliser N, a reduction in grain protein from 11% to 9% and a reduction in canopy N ratio due to reduced N in stem material (Sylvester-Bradley *et al*., 2010).

The challenge to produce high yielding, high protein, low optima bread wheats is greater than for feed wheats, due to the market requirement for 19.5 kg N / tonne grain having 13% protein. Unless market requirements for protein can be reduced, the only routes to reduced N requirements of bread wheats are through maximising recovery of soil and fertiliser N or maximising N harvest index.

Improving uptake of N from soil and fertiliser might be achievable through improved rooting (Foulkes *et al*., 2009). Greater rooting at depth may improve recovery of soil N, however this will not be quantitatively important in well-cropped soils, and it is unlikely that greater rooting in the topsoil would aid recovery of fertiliser N because topsoils are well explored by wheat roots (King *et al*., 2003). Indeed, it may be that recovery of fertiliser N (which largely resides in the topsoil throughout the crop's life) would be increased by reducing roots and associated carbon deposition near the surface, hence reducing the extensive immobilisation of fertiliser N that occurs during crop growth (King *et al*., 2001).

It has been reported that genetic modification of the gene for alanine amino-transferase can reduce N requirement in a range of species, including wheat and oilseed rape (Good *et al*., 2007). Whilst the physiological mechanisms at play in this manipulation are not yet fully explored, it seems that the modification increases sink demand for N, increasing recovery of N from soil and fertiliser. This raises interesting questions about the dynamics of N in crops (suggesting that N uptake is demand limited rather than constrained by rooting) and suggests additive opportunities may exist to optimise rooting, N immobilisation, N demand, N uptake, N assimilation, N conversion to dry matter and N partitioning to grain.

The introduction of symbiotic N fixation into wheat has long been a goal of plant scientists (Colebatch *et al*., 2002). However, it is worth noting some doubt here as to whether such a transformation would help in achieving a HYLO wheat type: N fixation is inherently energy demanding, thus will almost certainly require a significant supply of photosynthate; reduced yields seem inevitable compared to wheats that receive their N from external sources.

Detecting N Requirements of Varieties

For varieties with low N requirement to be detected in the market place, and indeed to stimulate the breeding of such varieties, variety testing regimes will need to distinguish N requirements of individual varieties, in the context of grain yield and other agronomic and quality characters. To do this properly would require testing of each variety in N response experiments with at least six N rates over many sites and years. Such wide-scale testing is likely to be prohibitively expensive, especially at early stages of varietal selection in breeding programmes and National Listing. Approaches are therefore required to test genetic differences in N optima in a cost effective way. This could be through use of existing or new measurements in testing trials, through altering the N rate used in testing trials or through testing at additional N rates. Measurements such as grain protein, N harvest index could be useful indicators of N requirement, as potentially could the use of canopy sensors detecting NDVI (Babar *et al*., 2006) or canopy temperature depression (Balota *et al*., 2007). The use of zero or reduced N rates in variety testing could be of benefit; varieties yielding well in these conditions may be likely to have lower N requirements. Testing with zero fertiliser N is likely to be valuable because it measures recovery of soil N, however, soil N levels should probably be moderate rather than low, to avoid the crop becoming highly N deficient, leading to very small varietal differences. Varieties which perform well due to improved fertiliser recovery are not distinguished at nil-N. N rates just below the optima may discriminate best between varieties in this respect. With two or three N rates it may be possible to predict N optima, especially if adjacent full N response experiments are conducted from which 'site' values for '*c*' and '*r*' parameters can be taken. Such an approach requires further validation.

Conclusions

Despite extensive previous work examining N efficiency, the widely adopted concept of NUE proves to be of limited value in achieving the improvements that growers, breeders, governments and society want. Rather, the aims of such work should be to reduce crop N requirement (or at least ensure that it is constrained) whilst grain yields are increased, and, in the case of bread making wheats, that protein content is maintained or improved. NUE and other efficiency indices, even NUEopt, are not effective targets for crop breeding because they are all increased by increasing yields, even if N optima actually increases too. They are also increased by reducing N optima with a much reduced yield. To ensure that the aims are met, it is vital that two measures are known separately; yield and N requirement. These cannot be reduced into a useful single metric; hence our adoption of the term High Yield, Low Optima – HYLO.

By analogy with other species (e.g. barley) it seems likely that HYLO wheats can be identified and developed, especially via the selection of varieties with low grain N%. However, progress towards a variety with much reduced N requirements (e.g. by 40%) will require screening of more traits and is likely to be gradual. The challenge to substantially reduce N requirements of bread wheats is much more difficult. Perhaps the best route to reduced N requirements here will be through working with the full bread supply chain to reduce protein requirements.

The successful introduction of HYLO varieties will depend crucially on there being an appropriate variety testing regime to show their value to growers and to give breeders clear targets.

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