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Published in:
Field Crops Research

DOI:
10.1016/j.fcr.2023.108829

First published: 01/03/2023

Document Version
Publisher's PDF, also known as Version of record

Field Crops Research 292 (2023) 108829

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Relative contribution of soil N availability and grain sink demand to the control of post-anthesis N uptake by field-grown spring barley

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ARTICLE INFO

Keywords:
Post-anthesis N uptake
Soil mineral N
Grain N demand
Degraining
Barley

ABSTRACT

Context: Increasing nitrogen (N) use efficiency of cereal production requires the optimisation of the source-sink relationships governing N accumulation by the grain, post-anthesis N uptake (PANU) and remobilization of N from vegetative tissue. There is evidence that N uptake may be regulated by both plant demand, of which grain N demand is the major component after flowering, and by N availability in the soil but the relative contribution of each to the control of PANU in field-grown barley crops is not understood.

Objective: The objectives were to investigate the control of PANU by determining its response to variations in N supply and grain N demand in crops differing in N status and soil mineral N content at flowering.

Methods: Field experiments were conducted on spring barley (*Hordeum vulgare* L.) over three years between 2011 and 2014. N fertilizer application at anthesis was used to vary post-anthesis N supply whilst partial degraining and barley variety were used to vary grain N demand. These treatments were imposed independently or in factorial combination depending on the experiment. Contrasting fertilizer regimes before or at the start of tillering, sites and seasonal weather generated a range of crop and SMN contents at flowering. Measurements were made of above-ground crop N content and soil mineral N (SMN) through the season.

Results: By, or shortly after, anthesis the SMN content of the root-zone had depleted to a relatively steady minimum value of around 60–115 kg N ha^{-1} depending on the site and year. PANU of control plants (non-degrained and without additional N fertilizer at anthesis) ranged from − 20–70 kg N ha^{-1}. Additional N fertilizer increased ($P < 0.05$) PANU in all experiments, whilst degraining reduced it significantly only in 2012. The response to degraining and anthesis N application were unaffected by crop N status at anthesis. There was no relationship between PANU and the unsatisfied grain N demand (that not met by retranslocation alone) at any level of anthesis N-fertilizer application in 2014. In 2012 there was a weak relationship accounting for only a small amount of the variation in PANU.

Conclusions: PANU of spring barley is limited mostly by N availability to the root system and not by a low grain N demand. High residual SMN contents at harvest and the poor relationship between SMN at anthesis and PANU suggest that transfer of N from bulk soil to the sites of active uptake at the root surface is a major limitation to PANU.

Implications: Increasing N uptake efficiency during grain filling will require improvements at the root-soil interface and not an increase in grain N demand.

1. Introduction

Crop growth has a large requirement for nitrogen (N). In the drive to increase crop productivity, global applications of N fertilizer rose by 40% between 2000 and 2020 with over 113 million tonnes now being applied annually (FAOSTAT, 2022). Cereals dominate the global production area and hence account for most of the N applied, but their efficiency of N use is low (Raun and Johnson, 1999). When N supply from the soil in the absence of fertilizer is accounted for, it has been estimated that on average as little as 30–40% of the N fertilizer applied to cereal crops globally is recovered in the grain (Raun and Johnson, 1999). Similar estimates can be calculated for UK grown wheat (Sylvester-Bradley and Kindred, 2009) and barley (Bingham et al., 2012). Inefficient use of N fertilizer poses serious and well reported risks for the environment. The high energy costs associated with its manufacture and the emission of nitrous oxide from soil following application contribute

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https://doi.org/10.1016/j.fcr.2023.108829
Received 12 September 2022; Received in revised form 6 January 2023; Accepted 15 January 2023
Available online 21 January 2023

to global warming (Dawson and Hilton, 2011; Rees et al., 2013). Surface run-off and leaching of nitrate from soil can lead to pollution and eutrophication of rivers and lakes (Ladha et al., 2005). In recognition of these problems, the EU has included an ambitious target of reducing fertilizer use by 20% over the next ten years in its strategy to improve the sustainability of its farming systems (European Comission, 2020). As crop yield and quality cannot be sacrificed in this process, it will require a significant and rapid improvement in fertilizer use efficiency and a greater use of biologically fixed N_2.

Opportunities exist for increasing the nitrogen use efficiency (NUE) of crop systems through changes in crop management and fertilizer practice (Ladha et al., 2005; Raun and Johnson, 1999) and through the breeding of more efficient varieties (Bingham et al., 2012; Hawkesford and Griffiths, 2019; Pask et al., 2012). However, progress is only likely to be made if a systems approach is taken in which proposed, and ultimately realised, improvements in individual components of the system are integrated and the synergies and potential trade-offs considered (Passioura, 2010; Raun and Johnson, 1999). A thorough understanding of what governs soil N availability and crop N demand is central to such an approach.

Growth of cereal crops can be considered in two main phases. In the first pre-anthesis phase, vegetative and then floral development lead to the growth of the canopy and yield bearing structures, whilst in the second post-anthesis phase net canopy growth ceases and grain filling associated with starch and protein deposition occurs (Miralles et al., 2021). The most rapid period of N uptake occurs pre-anthesis during stem extension. After anthesis, uptake of N declines, canopy senescence begins and a large-scale remobilization of N from vegetative tissue and translocation to the grain occurs (Barraclough et al., 2014; Hawkesford and Griffiths, 2019). At harvest maturity, large quantities of soil mineral N have been found to remain apparently unused (Bingham et al., 2012).

A number of crop traits have been identified that might improve NUE of cereals (Gaju et al., 2011, 2014; Le Gouis et al., 2016). There is considerable interest in the genetic improvement of N remobilization to the grain (Gaju et al., 2014, 2016). However, maximising N recovery from vegetative tissue and accumulation in the grain must be balanced against the potentially negative impact of early leaf senescence and reduction in photosynthetic activity on yield (Hawkesford and Griffiths, 2019). It must also be balanced against the potential effects on post-anthesis N uptake (PANU) and the nitrate residue remaining in soil at harvest. If satisfying grain N demand by increasing the efficiency of N remobilization leads to a reduction in PANU, the result might be a greater residual soil nitrate concentration and risk of leaching after harvest. Negative relationships between N remobilization and PANU have been highlighted (Triboi and Triboi-Blondell, 2002).

There is evidence that N uptake by root systems may be regulated by both plant N demand (Cooper and Clarkson, 1989; Garnett et al., 2013; Gojon et al., 2009; Imsande and Touraine, 1994) and N availability (Gooding et al., 2007; Sieling and Kage, 2021), but the relative importance of each is not understood especially for cereal crops during grain filling. Grain growth creates the largest demand for N post-anthesis as large amounts of N are required for the synthesis of structural and storage proteins (Matre et al., 2003). Mi et al. (2000) observed that PANU of wheat grown in pots was related to grain sink size. A genotype with long ears and large grain number had a greater PANU and was more responsive to N applications at anthesis compared to a short-eared counterpart. Moreover, PANU was reduced substantially in the long-eared genotype when grain N demand was reduced by spikelet removal. Deng et al. (2019) have also reported reduced N uptake by wheat following spikelet removal. Here the reduced grain demand resulted in a larger residual soil nitrate concentration and greater N_2O flux. In a comparison of the NUE of old and modern spring barley varieties, the N uptake efficiency (NUpE; N uptake/N supply) was greater in the higher yielding modern varieties (Bingham et al., 2012). NUpE was positively correlated with post-anthesis biomass gain and PANU and negatively correlated with the proportion of N captured before anthesis. It was hypothesised that the larger grain number formation of the higher yielding modern varieties generated a greater sink demand for N thereby increasing PANU.

Although N demand for grain growth represents the major sink for N after flowering, other sinks may contribute to the overall plant N demand that regulates PANU depending on the stage of development of the crop. Taulemesse et al. (2015) reported that in wheat, grown with non-limiting N supplies after flowering, the rate of uptake during early grain development was negatively related to the plant N status at flowering. Even when plant N status (nitrogen nutrition index) at flowering was comparable varieties differed in their N uptake during the early post-anthesis phase (Taulemesse et al., 2016). The authors suggested there was a negative regulation of uptake by an N satiety signal, independent of plant N status at flowering, possibly linked to N demand by stem growth during this period. Currently the signals and molecular mechanisms involved in the internal regulation of PANU in response to plant (including grain) N demand and N remobilization from vegetative organs are far from clear.

By contrast control of PANU by soil N availability is suggested by the increase in PANU and grain N concentrations often observed in response to applications of fertilizer N at or around anthesis (Gooding et al., 2007; Sieling and Kage, 2021). The response is consistent with the non-saturated kinetics of low affinity nitrate transporters (LATS) (Glass, 2003).

Whilst there is evidence to suggest that PANU may be controlled by both plant N demand during grain filling and soil N availability, the relative poise of the control in field-grown barley crops is not known with certainty. In particular, it is not clear whether the large residual soil mineral N contents observed in some crops at harvest is the result of a low plant (and more specifically grain) N demand or an inability of the root system to access the N. The aim of the experiments reported here was to investigate the relative contribution of N supply and grain sink demand to the control of PANU by field-grown spring barley. There were three specific objectives. The first was to determine the response of PANU to fertilizer N applied at anthesis and reductions in grain sink demand imposed independently by partially degraining the ear. The second was to establish whether the response of PANU to these treatments was influenced by plant N status and the soil mineral N content at flowering. The third was to determine whether altering grain number through variety or degraining altered the response of PANU to N supply. In field experiments conducted over three years we show that PANU is controlled mostly by soil N availability rather than a limited grain N demand.

2. Materials and methods

2.1. Site conditions and general husbandry

Field experiments were conducted under rainfed conditions at SRUC trial sites near Edinburgh, UK over three years, 2011, 2012 and 2014. In 2011 the experiment was located at Boghall Farm, Midlothian (latitude 55.878 °N, longitude 3.198 °W) on a sandy loam soil of the Duncrahill series with an organic matter content of 6.9% and topsoil pH of 6.3. Crops in the preceding three years were all spring barley. In 2012 and 2014, experiments were located at Cauldshiel Farm, East Lothian (55.881°N, 2.835°W). Here the soil was a sandy loam of the Humbie series with an organic matter content of 4.2% and pH 6.3. In each case the previous crop was spring barley with winter wheat and winter oilseed rape before that.

Seedbed preparation followed ploughing and plots (10×2 m) of two row spring barley (*Hordeum vulgare;* cv dependent on experiment detailed below) were sown at a rate of 350–360 viable seeds m^{-2} on the 21st, 9th and 19th March in 2011, 2012 and 2014 respectively. Hereafter, these are referred to as drilled plots to distinguish them from the aggregates of plots that make up the main plots in the experimental designs described below. P and K fertilizer was applied as a top dressing

to the soil within a week after sowing according to soil analysis. Fertilizer N was applied as ammonium nitrate granules at rates and timings dependent on the experimental treatment (described below). Manganese and sulphur and a robust crop protection programme were applied according to local commercial practice to avoid trace element deficiencies and to control weeds, pests, and diseases. As genotypes selected for investigation in 2014 included some old tall varieties, trinexapac-ethyl (Moddus, Syngenta, 0.4 l ha^{-1}) and ethephon (Cerone, Nufarm UK, 0.5 l ha^{-1}) were applied at Zadoks growth stage 31 and 37 (Tottman, 1987) respectively to prevent lodging. No growth regulators were applied in 2011 and 2012.

2.2. 2011 and 2012 experiments

2.2.1. Experimental design and treatments

Experiments were a factorial combination of N fertilizer dose, barley variety and anthesis treatment laid out in a randomised split-split plot design with four replicate blocks. In 2011, two N fertilizer levels, zero and full N (130 kg ha^{-1}, the recommended rate for malting barley crops) were randomised in main plots; two varieties, Westminster and Optic, were randomised within sub plots; and three levels of anthesis treatment, additional N fertilizer, partial degraining and controls (intact plants without additional fertilizer) were applied to sub-sub plots. Guard areas between the main plots enabled N to be applied by tractor 42 days after sowing at GS22 without contaminating neighbouring plots. The experimental unit at the sub-plot level (an individual combination of N fertilizer and variety) comprised of two adjacent drilled plots; one was designated for measurement of canopy light interception, combine harvesting and yield measurement, the other for destructive sampling during the season. Anthesis treatments were imposed when main shoot ears had emerged (GS59). Plants within the third row from the edge of the plot were selected for degraining. Canes were used to mark either end of a 0.5 m length of the row and the upper half of all emerged ears in the row were excised and discarded. This treatment also removed part of the awns of the remaining grains. An equal length of the third row on the opposite side of the plot was marked, but ears were left intact to serve as controls. Degrained and control rows were located in the combining plots. Within the sampling plots, a 1 m^2 area adjacent to the degrained and control rows in the combining plots was marked with canes (avoiding the outer 0.25 m of the plot) and a granular formulation of ammonium nitrate applied uniformly by hand to the spaces between rows at a rate equivalent to 40 kg N ha^{-1}; this was the additional N treatment. To ensure uptake of N was via the root system, the fertilizer was applied when the crop was dry and plant rows were gently parted to facilitate application to the soil surface. Plants were shaken gently after application to dislodge any granules that may have fallen on the leaves.

The 2012 experiment followed the same design except for the inclusion of a third level of N fertilizer (80 kg ha^{-1}, referred to as low N) in the main plots. Here N was applied 10 days after sowing, prior to crop emergence. To avoid a repeat of the problems of large sample variability encountered in 2011, the size of the anthesis treatment and sampling areas was increased. This was accommodated by having three drilled plots per experimental unit; one for degraining treatments and combine harvesting, one for frequent destructive sampling and the third for anthesis N fertilizer application. Degraining was imposed as described above on two 0.5 m row lengths (third and fourth row from the plot edge) at each of two locations per plot. These locations were at diagonally opposite ends of the drilled plot, in areas where plant growth was representative of the plot as a whole. Row lengths of intact control plants were marked at either end of the opposite diagonal. Fertilizer N (40 kg ha^{-1}) was applied as described above to two 1×2 m areas, one in each half of the designated drilled plot. The target growth stage for the 'anthesis' treatments was GS59, but weather conditions delayed their imposition until the start of grain filling (GS71/73). After degraining, rows of cut ears and control plants were misted with fungicide (prothioconazole plus tebuconazole; Prosaro, Bayer Crop Science at a concentration of 5 ml l^{-1} of water) to prevent fusarium infection.

2.2.2. Soil measurements

Soils were sampled from the designated sampling plots at two to three-week intervals after sowing. Soil cores were taken to a depth of 80 cm using a 2.5 cm diameter auger, separated into three depth intervals, 0–30, 30–60 and 60–80 cm, sealed in polythene bags and frozen at −20°C immediately on return to the laboratory. Any granules of undissolved N fertilizer were cleared away from the around the sampling point before coring. In 2012 an initial pre crop emergence sample was taken at depths of 0–30 cm and 30–80 cm at five locations across the experimental blocks and combined for analysis.

For determination of soil mineral N concentrations, 10 g of thawed and well mixed soil was extracted in 50 ml of 1 M KCl for 1 h, centrifuged to settle out soil particles and the ammonium and nitrate concentration of the supernatant quantified on a segmented flow autoanalyser (Skalar Analytical BV, The Netherlands). Freezing and thawing of soil prior to determination of ammonium and nitrate has been shown to alter the ratio of these ions compared to extracts from fresh soil, but has little effect on their sum (total soil mineral N, SMN) (Kindred et al., 2012). Gravimetric soil moisture content was determined after drying at 105 °C until constant weight. After harvest soil dry bulk density was determined at soil depths of 10–15, 25–30, 45–50 and 70–75 cm (Rowell, 1994). At each depth, two samples were taken from each of two locations per site.

2.2.3. Plant sampling and measurement

Canopy area index (CAI; includes ear, stem and leaf tissue) and photosynthetically active radiation interception was measured at GS55 on plots designated for combine harvesting using a Sunscan canopy analysis system (Delta T Devices, Cambridge, UK). Measurements were taken at an angle of 45° to the plant rows at five points per plot.

At two to three-week intervals after N fertilizer application, plant samples were taken from a 0.5 m row length at diagonally opposite ends of the designated sampling plots. The outer two rows and plants within 0.5 m of the plot ends were not sampled. Plants were pulled from the soil, the roots excised and discarded, and shoots placed in polythene bags to prevent moisture loss. Samples were stored at 4 °C for no longer than 12 h prior to processing. Shoots were separated into the following fractions: leaf laminae, leaf sheath plus stem and after its emergence, the ear. Each fraction was dried at 80 °C for 48 h and weighed.

Immediately prior to harvest the marked row lengths of intact and degrained plants, and equivalent row lengths of plants in subplots supplied with anthesis N fertilizer, were sampled as described above. The number of degrained and intact ears were counted plus the number of ear-bearing and non-ear-bearing shoots. Shoots were separated into ears, leaf laminae and stem plus leaf sheath fractions, and the tissue dried and weighed as above. Ears were threshed with a laboratory thresher (Wintersteiger LD 180, Austria), the grain and chaff were recovered, and the grains weighed. Chaff weight was calculated as ear weight minus grain weight. Chaff was added to the straw fraction (leaf, leaf sheath plus stem) for analysis of tissue N concentration. Plant tissue from the different fractions was initially coarse milled and then ball milled into a fine powder. Tissue N concentration was determined by Dumas combustion in a Flash 2000 elemental analyser (Thermo Scientific, UK). Plots were combine harvested for yield determination and a sample of grain taken for measurement of mean grain weight and gravimetric moisture content.

2.3. 2014 experiment

2.3.1. Experimental design and treatments

In 2014 the experiment was a factorial combination of genotype, anthesis N and degraining treatments in a split-split plot randomised block design with four replicate blocks. Here genotype was randomised within main plots, anthesis fertilizer N within sub plots and degraining within sub sub plots. Six genotypes (Kenia, Carlsberg, Zephyr, Aramir,

Optic and Westminster) were selected to give a range of old and modern varieties with dates of commercial introduction ranging from 1931 (Kenia) to 2002 (Westminster) (Bingham et al., 2012). There were three levels of anthesis N (0, 40 and 80 kg N ha^{-1}) and two levels of degraining (partial degraining and no degraining). All plots received a basal N fertilizer supply of 120 kg N ha^{-1} split in two halves, the first five days after sowing and the second 35 days after sowing at GS13. Each 10 m long drilled plot was divided into 4×2 m length sub plots, with 0.5 m gaps between sub plots and a 0.25 m guard at each plot end. Anthesis N treatments were randomised across three of the sub plots, the remaining sub plot was used for plant and soil sampling at anthesis. Within each anthesis N sub plot, adjacent 0.75 m lengths of the third and fourth row of plants were marked with canes and used for degraining; equivalent lengths of rows 7–12 were reserved for controls. The side of the plot from which rows were counted, and hence the position of degrained treatments and controls, was randomised between sub plots. Anthesis N and degraining treatments were applied seven days after GS55 (approximately GS69/71) as described above. Anthesis N was applied to the entire sub plot area. After degraining, cut ears and control plants were treated with fungicide (details above).

As an additional measure to prevent lodging, support netting was erected at GS31 over each anthesis N sub plot. Plastic pea netting (20 cm square mesh) was stretched horizontally 0.5 m above the soil surface and secured to wooden stakes in each corner. During stem extension, the barley plants grew through and above the mesh support.

2.3.2. Plant measurements

Canopy area index and photosynthetically active radiation interception was measured at GS55 on sampling plots using a Sunscan canopy analysis system (Delta T Devices, Cambridge, UK) as described above. Two adjacent 0.5 m row lengths were then sampled from each end of the plot avoiding the outer two rows and areas within 0.5 m of the ends. Plants were pulled from the soil, the roots excised and discarded, and shoots placed ear first into polythene bags to prevent moisture loss and transferred to the laboratory for assessment. Samples were processed immediately or stored in their bags at 4 °C in the dark for no longer than 48 h before assessment. Each sample was weighed fresh, and the number of ear-bearing and non-ear-bearing shoots recorded. Shoots were then divided at random into four subsamples. One representative subsample was weighed fresh, shoots were separated into ears, leaf laminae, and stem plus leaf sheaths fractions, dried for 48 h at 80 °C in a fan-assisted oven and each fraction weighed to the nearest 0.01 g.

Plants were harvested when the majority of ears were at GS91. Some genotypes and anthesis treatments had a significant number of green immature shoots at this time. The support netting was gently cut from around the degrained plants taking care to minimise the shedding of leaf tissue. Canes (0.5 m long) were placed either side of rows 3 and 4 and the central 0.5 m of the degrained rows sampled. Plants were pulled from the ground, roots removed and discarded, and shoots sealed in polythene bags. Equivalent lengths of rows 7–12 were similarly sampled as control plants. The positioning of these sampling areas was such that sampled plants were no less than 0.25 m from the edge of the plot and 1.25 m from neighbouring sub plots.

Control plants were weighed fresh and a 25% subsample by weight taken. The sub sample was divided into green and non-green (mature) shoots. The number of ear-bearing and non-ear-bearing shoots in each was recorded. Ears were excised at the collar and ears and remaining shoot tissue dried at 80 °C for 48 h for dry weight determination. Samples of degrained plants were processed in the same way, but without subsampling as the sample size was smaller. Additionally, for mature non-green shoots, the number of degrained (trimmed) ears and the number that were missed during degraining, was recorded. Ears were threshed with a laboratory thresher (Wintersteiger LD 180, Austria) and the chaff retained and added to the rest of the shoot fraction for determination of tissue N. Grains were weighed and the number in each sample was counted manually. Plant tissues were milled, and their N concentrations determined as described above.

2.3.3. Soil measurements

After plant sampling at anthesis soils were sampled from the designated sampling sub plot for determination of mineral N. Soil cores were taken to a depth of 60 cm using a 2.5 cm diameter auger, separated into two depth intervals, 0–30 and 30–60 cm and stored in polythene bags at 4 °C for a maximum of three days prior to analysis. Soil mineral N concentrations were determined as described above.

2.4. Meteorological data

Monthly rainfall and average daily temperatures for Penicuik were collated from Anon (2022) and the UK Met Office (2022). Penicuik is located four miles from the experimental site in 2011 and 34 miles in 2012 and 2014.

2.5. Calculations & statistical analysis

Yields and mean grain weights (MGW) are expressed on a 100% dry matter basis. Grain numbers m^{-2} were calculated by dividing the yield by the MGW. Soil mineral N (SMN) content to 60 cm was calculated from measurements of nitrate and ammonium concentrations in each soil layer and expressed per ha after adjusting for soil dry bulk density in the topsoil and subsoil. Soil N supply (SNS) was calculated as an index of the crop available N by summing SMN and above ground crop N content (N offtake; N_{off}) at each sampling date. Post-anthesis N uptake (PANU) was determined as the difference in crop N offtake at anthesis and final harvest. For degrained plants, PANU was corrected for the N removed from the ear by degraining. To estimate the maximum N content at saturation of mature grains in 2014, the grain N content (mg N $grain^{-1}$) was plotted against the amount of N available for grain filling. The latter was estimated as:

$$\text{N available for grain filling} = \text{ear } N_{anth} + N_{rem} + \text{soil } N_{anth} + \text{anthesis } N_{fert} \text{ uptake}$$

where ear N_{anth} is the N content of ears at anthesis, N_{rem} is the N potentially remobilized from leaf and stem tissue, soil N_{anth} is the SMN at anthesis potentially available for uptake and anthesis N_{fert} uptake is the uptake of additional fertilizer N applied at anthesis. N_{rem} was estimated as the product of the leaf plus stem N content at anthesis and a variety specific value for remobilization efficiency. The latter was taken be to that observed for intact (control) plants given no additional N fertilizer at anthesis and is the fraction of N that was present in leaf and stem at anthesis that was not present at harvest (Pask et al., 2012). Soil N_{anth} was considered to be the PANU of intact plants measured in the absence of additional anthesis fertilizer. Anthesis N_{fert} uptake was estimated as the product of the amount of N applied and an assumed fertilizer recovery of 0.6 (Kendall et al., 2021; Sieling and Kage, 2021). Values of N available for grain filling were expressed per unit grain number. The unsatisfied grain N demand for a given variety was then calculated as the difference in the N content per grain at saturation and the amount that can be supplied solely from remobilization. The N content at saturation was taken to be the N content per grain for mature grains of degrained plants supplied with 80 kg N at anthesis. Evidence is presented in the results to show that this combination of treatments did result in near N saturation of mature grain.

The analysis of the relationship between unsatisfied grain N demand and PANU developed using 2014 data was applied to data from 2012. In the absence of any direct measurement of grain N content at saturation for the different pre-anthesis fertilizer regimes in 2012, the unsatisfied grain N demand was calculated using values of N saturation estimated in two different ways from 2014 data. Firstly, the N content at saturation for Optic and Westminster measured under the full pre-anthesis fertilizer regime in 2014 was used. This assumes that the N capacity per grain is a

fixed characteristic of the variety and is unaffected by year and fertilizer regime in 2012. Secondly, the N% at saturation from 2014 was used to estimate saturation N contents for crops under different fertilizer regimes and with differing mean grain weights in 2012. This assumes that the N% at saturation is a fixed characteristic of the variety rather than N content and is unaffected by effects of year and fertilizer regime on potential grain weight. Statistical significance of treatment effects on crop characteristics at anthesis, final yield and yield components were determined by analysis of variance (anova). Repeated measures anova was used to analyse treatment effects on crop and soil N dynamics in 2011 and 2012 experiments. Model II regression by groups was used to investigate the relationship between unsatisfied grain N demand and PANU. All routines were carried out in Genstat 19th edition (VSN International Ltd, UK). Residuals were checked for normality of distribution and homogeneity of variance and data transformed where necessary.

3. Results

3.1. Weather

Seasonal rainfall differed widely between the experimental years. 2012 was an exceptionally wet year with rainfall between April and July being over two to three times the long-term average (LTA) for the region (Fig. 1). By contrast, rainfall in 2011 and 2014 were closer to the LTA. In 2011 a drier than average spring (March to April) was followed by a wetter than average summer (May to August). In 2014, April and May were wetter than average, but from June to August rainfall deviated from the LTA by less than 40%. Average daily temperature differed relatively little from the LTA in each of the experimental years.

3.2. Experiments 2011 and 2012

3.2.1. Crop growth and yield

Fertilizer N regime had a significant effect on crop growth when measured at anthesis (Table 1). Averaged across varieties above ground biomass, N content (N offtake), tissue N concentration (N%) canopy area index (CAI) and the fraction of incident PAR intercepted were all increased ($P \leq 0.03$) in crops with fertilizer compared to those without fertilizer. These effects were observed in both 2011 and 2012. In 2012, canopy size (CAI), PAR interception, tissue N% and N offtake were all considerably lower than those observed in 2011. In each year, there was a significant main effect of variety on biomass and CAI with cv Westminster producing a larger (8–25%) canopy than Optic (Table 1). The only fertilizer x variety interaction observed was for CAI in 2012 where Westminster responded to N fertilizer with a larger increase in CAI than Optic ($P = 0.005$).

Not surprisingly, grain yields were significantly greater in crops given N fertilizer than in unfertilized crops (Table 2). The greater yields were associated with a larger grain number m^{-2} and mean grain weight (MGW) and were observed in both 2011 and 2012. Yields in 2012 were around 44% lower in 2012 compared to 2011, both with and without N fertilizer. The low yields were associated with low solar radiation receipts resulting from the long period of dull wet weather. A smaller grain number contributed most to the lower yield, but MGW was also around 18% lower. Averaged over fertilizer treatments yields of Westminster and Optic did not differ in 2011, but in 2012 the yield of Westminster was 18% greater than Optic ($P < 0.001$); the result of a larger grain number ($P = 0.057$) and MGW ($P < 0.001$). Interactions between fertilizer treatment and variety on yield and its components were generally weak or absent and inconsistent between years.

Fertilizer regime significantly ($P = 0.006$) influenced grain N% in 2011. The full N regime resulted in a grain N concentration, averaged across varieties, of 1.59%, whilst N concentration in the zero N regime was just 1.02% (Table 2). There was a weak ($P = 0.066$) effect of variety but no significant interaction between fertilizer regime and variety. In 2012 fertilizer regime had no effect on grain N concentration with each regime resulting in a concentration as low as the non-fertilized plots in 2011 (~1.0% N).

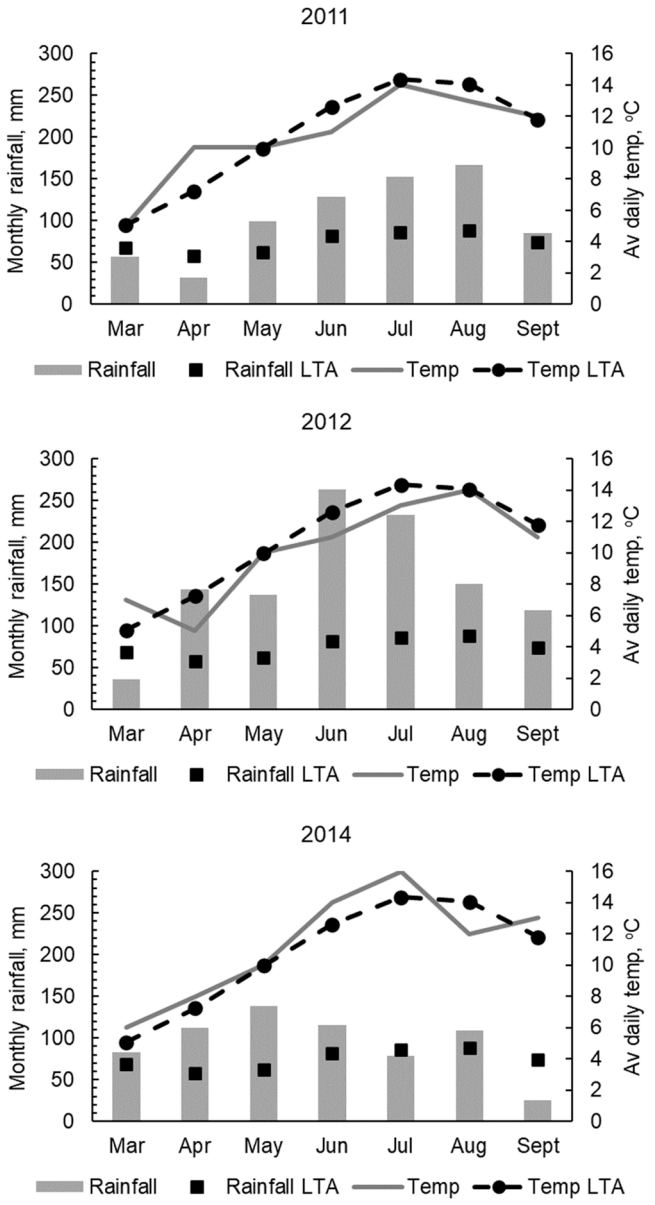

Fig. 1. Monthly rainfall and average daily temperature for the growing season in experimental years 2011, 2012 and 2014. The long-term average (LTA, 1991–2020) for the area are shown for comparison.

3.2.2. Soil mineral N and crop N dynamics

There was no significant effect of variety on soil mineral N (SMN), nor a variety x time or variety x fertilizer interaction in either year (Supplementary data Table S1). Although there were weak interactions between fertilizer and variety on crop N offtake, these were not altered over time. Thus, data presented for SMN and crop N offtake over time are averaged across the two varieties. In 2011 soil mineral N (SMN) in the top 60 cm of the soil profile at around the time of crop emergence and before application of fertilizer was 65 kg ha^{-1} (Fig. 2). After application of fertilizer the SMN rose to 415 kg N ha^{-1}, the increase exceeding the 130 kg of N applied. SMN then declined reaching values close to those of non-fertilized soils six days after anthesis. There was relatively little change in SMN during the grain filling period. The depletion in SMN coincided with a steady increase in crop N

Table 1
Effects of fertilizer treatments and variety on above ground biomass, N offtake (above-ground crop N content), tissue N concentration (N% dry weight), canopy area index (CAI) and the fraction of incident PAR intercepted by the canopy at anthesis in 2011 and 2012.

		2011					2012				
Fertilizer N	Variety	Biomass, t ha^{-1}	Noff, kg ha^{-1}	N%	CAI	Frac PAR interception	Biomass, t ha^{-1}	Noff, kg ha^{-1}	N%	CAI	Frac PAR interception
Full	Optic	10.87	157.2	1.44	4.4	0.94	11.53	91.3	0.79	2.7	0.83
Low	Optic						8.68	58.3	0.67	2.3	0.78
Zero	Optic	6.79	54.1	0.79	1.4	0.64	3.31	22.5	0.68	1.0	0.52
Full	Westminster	12.88	189.7	1.48	4.8	0.95	11.81	89.3	0.76	3.4	0.88
Low	Westminster						9.91	67.0	0.68	3.0	0.86
Zero	Westminster	7.86	62.2	0.78	1.6	0.68	3.75	25.1	0.67	1.2	0.56
Mean Fert	Full	11.88	173.4	1.46	4.6	0.94	11.67	90.3	0.77	3.0	0.86
	Low						9.30	62.6	0.67	2.6	0.82
	Zero	7.33	58.1	0.79	1.5	0.66	3.53	23.8	0.67	1.1	0.54
Mean Var	Optic	8.83	105.6	1.12	2.9	0.79	7.84	57.4	0.71	2.0	0.71
	Westminster	10.37	126.0	1.13	3.2	0.81	8.49	60.5	0.70	2.5	0.77
P value	Fert	0.003	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Var	0.032	0.115	0.793	0.023	0.010	0.038	0.132	0.352	< 0.001	< 0.001
	Fert*Var	0.429	0.314	0.688	0.373	0.125	0.351	0.118	0.548	0.005	0.106
LSD (5%)	Fert	1.674	14.28	0.146	0.66	0.049	2.129	18.20	0.037	0.43	0.039
	Fert*Var	ns	ns	ns	ns	ns	ns	ns	ns	0.43	ns

Table 2
Effects of N treatments and variety on grain yield, yield components and grain N concentration (% dry weight) in 2011 and 2012.

		2011					2012			
Fertilizer N	Variety	[1]Yield, t ha^{-1}	[1]Grains m^{-2}		MGW, mg	N%	Yield, t ha^{-1}	Grains m^{-2}	MGW,mg	N%
Full	Optic	6.38	15075	a	42.31	1.50	3.29	9545	34.39	1.04
Low	Optic						2.67	8158	32.66	1.01
Zero	Optic	2.17	5619	c	38.57	1.00	1.17	3698	31.37	1.00
Full	Westminster	6.21	13343	b	46.54	1.69	3.90	10163	38.27	1.13
Low	Westminster						3.21	8675	36.95	1.08
Zero	Westminster	2.24	5668	c	39.55	1.05	1.32	3917	33.61	0.98
Mean Fert	Full	6.29	14183		44.42	1.59	3.60	9854	36.33	1.08
	Low						2.94	8417	34.81	1.04
	Zero	2.21	5643		39.06	1.02	1.24	3807	32.49	0.99
Mean Var	Optic	3.84	9203		40.44	1.25	2.38	7134	32.81	1.01
	Westminster	3.84	8697		43.04	1.37	2.81	7585	36.28	1.06
P value	Fert	< 0.001	< 0.001		0.003	0.006	< 0.001	< 0.001	< 0.001	0.082
	Var	0.996	0.032		0.054	0.066	< 0.001	0.057	< 0.001	0.125
	Fert[1]Var	0.218	0.018		0.185	0.218	0.051	0.724	0.04	0.362
LSD (5%)	Fert						0.329	717.9	1.207	ns
	Fert[1]Var	ns			ns	ns	0.351	ns	1.324	ns

[1] Data transformed log10 (x + 1) prior to analysis. Back transformed means shown. For grains m−2 in 2011, means followed by a different letter in the Fert*Var interaction are significantly different (P = 0.05)

accumulation reaching a value of 173 kg N ha^{-1} around anthesis. There was little net accumulation by the crop between anthesis and harvest. The SMN of soils without N fertilizer varied by less than 63 kg N ha^{-1} over the course of the experiment ($P > 0.05$). Crop N uptake followed dynamics comparable to those of fertilized crops, reaching a maximum value of N offtake around anthesis with no significant net accumulation thereafter. The N offtake at harvest was just 24% of that of fertilized crops (Fig. 2).

The SNS is an index of the total amount of N in the crop-soil system, in the form of N in plant tissue and mineral N (nitrate plus ammonium) in soil, at any point through the season. The reference values are the sum of SMN at crop emergence in the absence of fertilizer and the amount of fertilizer N applied and represent what is available for crop uptake if there is no subsequent net mineralization or loss of N. After fertilizer application SNS rose to a value of around 400 kg N ha^{-1} between 51 and 79 days after sowing (DAS). Examination of the 95% confidence intervals indicated that SNS for these sample times was significantly greater than the reference value. Thereafter SNS declined to values after anthesis that remained significantly greater ($P < 0.05$) than the SMN plus fertilizer N reference. From 51 DAS onwards the SNS of non-fertilized plots was on average 40 kg N ha^{-1} greater than the SMN reference measured around the time of crop emergence, but for the most part the SNS did not differ significantly ($P > 0.05$) from the reference.

In 2012, at 20 DAS (around the time of crop emergence) the SMN of non-fertilized plots was 131 kg N ha^{-1} (Fig. 3); double that in 2011 (Fig. 2). With full and low rate fertilizer application the SMN was ~452 kg N ha^{-1}. There was a small (36%) but not statistically significant ($P > 0.05$) depletion of SMN from non-fertilized plots between 20 and 82 DAS and a large (80%; $P < 0.05$) depletion from plots given the full and low rates of N fertilizer over the same period. By 80 DAS SMN in fertilized plots had been depleted to levels found in non-fertilized plots. Thereafter, SMN remained largely unchanged in each treatment. This point was reached considerably earlier in 2012 (32 days before anthesis) compared to 2011 (~6 days after anthesis).

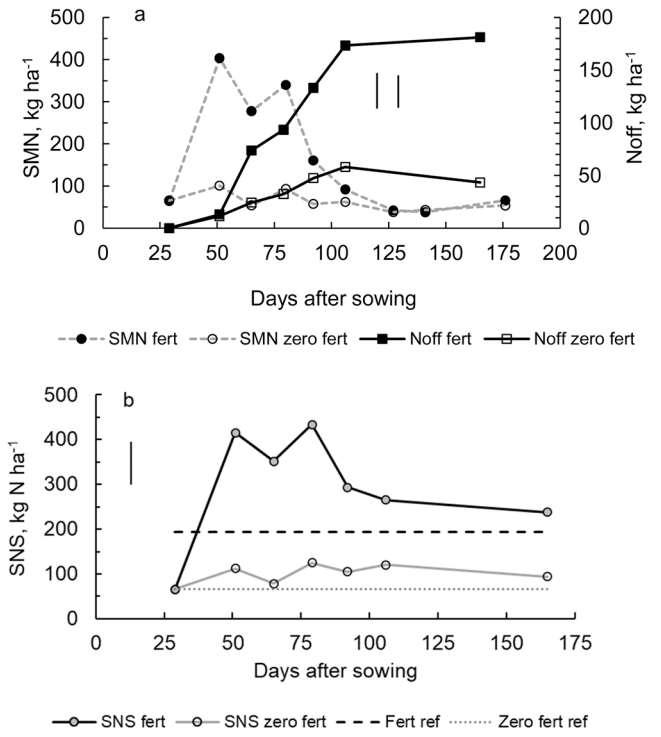

Fig. 2. Changes in a) soil mineral N (SMN) content to 60 cm and crop N offtake over time for crops in 2011 supplied with and without N fertilizer; vertical bars represent LSD (5%) for the time x fertilizer interaction for SMN (left) and crop N offtake (right); b) Soil N Supply (SNS; sum of SMN and N offtake); vertical bar represents LSD (5%) for the time x fertilizer interaction. Horizontal lines in b) are reference values for SNS calculated as SMN at the first sampling on non-fertilized plots before crop N uptake plus amount of N fertilizer applied. Fertilizer was applied 42 days after sowing (DAS) and anthesis was 100 DAS.

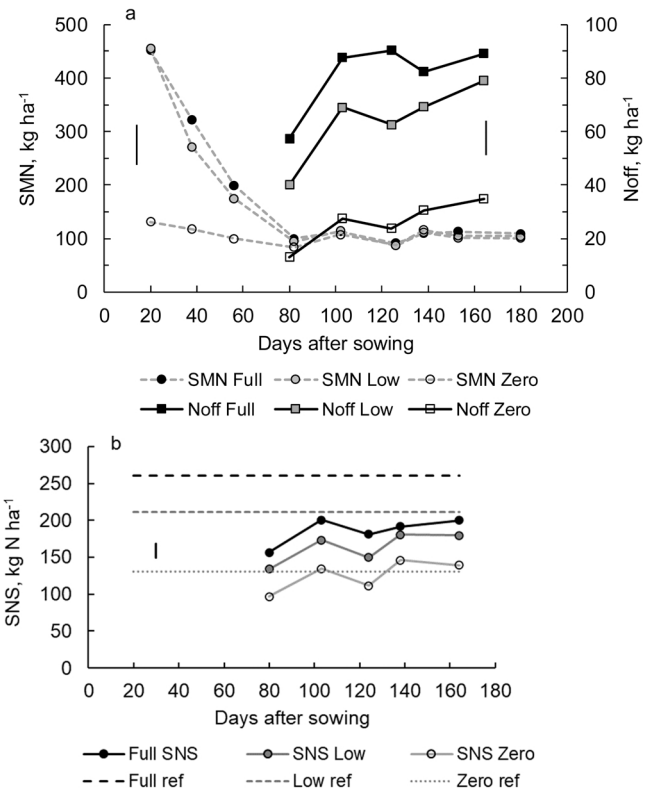

Fig. 3. Changes in a) soil mineral N (SMN) content to 60 cm and crop N offtake over time for crops in 2012 supplied with full, low and zero N fertilizer; vertical bars represent LSD (5%) for the time x fertilizer interaction for SMN (left) and crop N offtake (right); b) Soil N Supply (SNS; sum of SMN and N offtake); vertical bar represents LSD (5%) for the time x fertilizer interaction. Horizontal lines in b) are for reference values for SNS calculated as SMN at the first soil sampling measured on non-fertilized plots before crop N uptake plus the amount of N fertilizer applied. Fertilizer was applied 10 days after sowing (DAS) and anthesis was 112 DAS.

Crop N offtake measurements were started 80 DAS. There was a significant ($P < 0.001$) effect of fertilizer treatment and time ($P < 0.001$) on N offtake, but no fertilizer x time interaction ($P = 0.099$). Thus, by the time measurements commenced fertilizer treatments had resulted in significant differences in crop N accumulation. From 80 DAS onwards these differences were largely maintained. Under each fertilizer regime, N offtake increased by 52–111% between 80 and 103 DAS, but thereafter (i.e. after anthesis) increased less or remained relatively unchanged (2–26%).

Under all fertilizer N regimes SNS increased between 80 and 103 DAS, but remained relatively constant during grain filling. During grain filling SNS of the full and low N fertilizer plots was significantly ($P < 0.05$) lower than their SMN plus applied fertilizer reference values. By contrast, the non-fertilized plots deviated little ($P > 0.05$) from the reference value over this period.

3.2.3. *Post-anthesis N uptake*

Post-anthesis N uptake was significantly influenced by fertilizer regime and by the application of additional treatments at anthesis in both 2011 and 2012 (Table S2). There was no significant effect of variety on PANU and no interactions between fertilizer regime, anthesis treatments and variety in either year. Means for the N fertilizer regime x anthesis treatment interaction are presented in Fig. 4. In 2011 there was large variability in measurements of PANU. Crops given the full N regime pre-anthesis had significantly greater PANU than unfertilized crops. PANU was increased by application of additional fertilizer at anthesis. This increase was 53 kg ha^{-1} when averaged over pre-anthesis N fertilizer regimes. Relative to controls partial degraining reduced PANU, but the effect was small (8.7 kg ha^{-1} averaged over fertilizer regimes) and not statistically significant. Negative values of PANU indicate apparent net losses of N from the above-ground biomass during grain filling. In 2012 PANU of crops given the full N fertilizer regime was significantly lower than those given low or zero N when averaged over the anthesis N treatments (Fig. 4b, Table S2). Application of additional N at anthesis increased PANU and degraining reduced PANU relative to controls. The lack of interaction between fertilizer N regime and anthesis treatment in both 2011 and 2012 indicates that the pre-anthesis fertilizer N regime did not alter the response of PANU to anthesis treatment in either year.

Using the grain number m^{-2}, the amount of N in the crop at anthesis available for retranslocation to grains plus estimated values of the N content per grain of the variety at near saturation, it was possible to calculate the unsatisfied grain N demand. This was the difference in the amount of N needed to saturate all grains and the quantity that can be supplied solely from remobilization. The results must be interpreted cautiously because they are based on estimates of N content per grain at saturation rather than measured values. However, two methods for estimating these values gave broadly comparable results. In the first, unsatisfied grain N demand was estimated assuming a fixed value of N content per grain for each variety at saturation. There was a weak positive relationship ($P < 0.05$, R^2 0.25) between PANU and unsatisfied grain N demand when the latter varied with degraining, variety and pre-anthesis fertilizer regime in plants given no additional N at anthesis (Fig. 5). Application of N at anthesis increased PANU significantly over the whole range of unsatisfied grain N demand associated with non-degrained controls. The second method for estimating unsatisfied grain N demand, which assumed a fixed value of grain N% for each

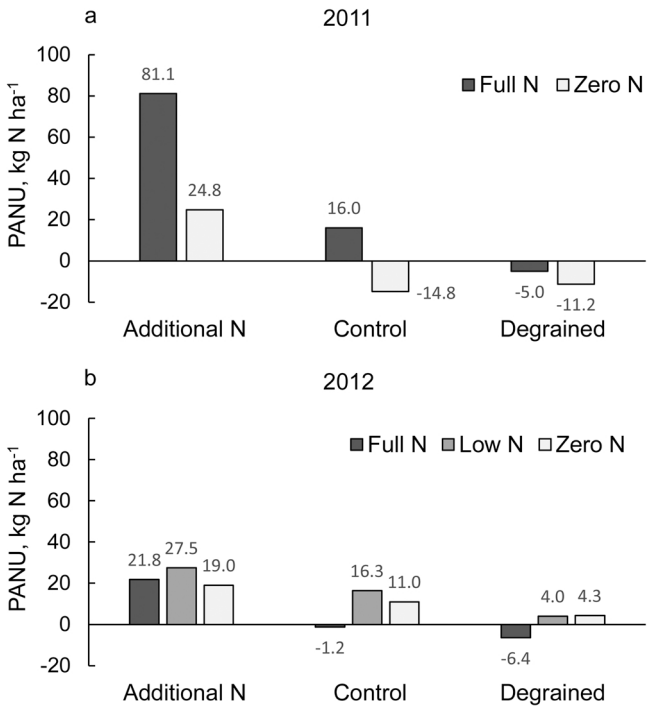

Fig. 4. Effects of pre-anthesis N fertilizer regime and anthesis treatments on PANU in a) 2011 and b) 2012. Values above the columns are means for the treatment combination averaged across varieties. LSD (5%) for the N regime x anthesis treatment interaction for 2011 is 43.1 except when comparing means within the same level of fertilizer regime when it is 49.7; for 2012 LSDs (5%) are 10.5 and 11.1 respectively. For N regime means averaged over anthesis N treatments, the LSDs are 23.7 (in 2011) and 6.5 (in 2012).

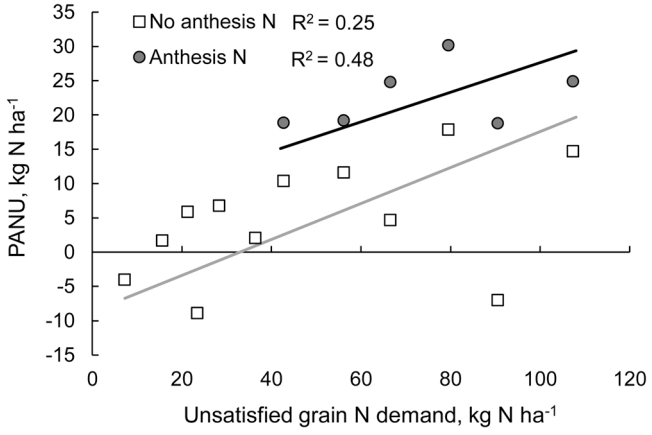

Fig. 5. Relationship between grain N demand that cannot be satisfied by remobilization of N from vegetative tissue and PANU for crops in 2012. For plants given no additional N fertilizer at anthesis each point is the mean of an individual variety x pre-anthesis fertilizer regime x degraining treatment combination. For plants given anthesis fertilizer N points are means of individual variety*pre-anthesis fertilizer combinations. Lines were fitted following model II regression with groups. Slopes were significantly different from zero ($P < 0.05$) but not different between groups ($P = 0.897$). Elevations of lines (constants) differed significantly between groups ($P < 0.001$). Unsatisfied grain N demand was estimated using fixed values of N content per grain at saturation measured in 2014 (method 1).

variety at saturation irrespective of the mean grain weight, gave lower values than the first. Nevertheless, there was a similar weak positive relationship between unsatisfied grain N demand and PANU with this method in the absence of anthesis N fertilizer ($P < 0.05$, R^2 0.28, data not shown). The large variability in PANU in 2011 and restricted number of treatments precluded the application of this analysis to 2011 data.

3.3. Experiment 2014

3.3.1. Yield and yield components

In 2014 there was an appreciable amount of later tillering such that some shoots were still green and had incompletely filled grains when the grains on main shoots and early formed tillers were mature and ready for harvest. Yield and yield components have, therefore, been estimated for the combined mature and immature shoots (total shoots) and for each group separately (Table 3 and Table S3). There were significant main effects of variety on yield, grain number m^{-2} and mean grain weight (MGW) with the total yield and MGW of older varieties tending to be lower than those of the more recent varieties Optic and Westminster (Table 3). Yields of non-degrained control plants without additional N fertilizer ranged from 5.82 t ha^{-1} for Carlsberg to 7.89 for Optic and grain numbers from 13983 to 18189 m^{-2} for the same varieties (Table S4). Immature shoots contributed 4–13% to the total yield of these control plants depending on the variety, but there was no clear association between late tillering and the date of introduction of the variety (Table S4). Not surprisingly, degraining reduced grain numbers m^{-2} and thus yield by approximately 35% through its effects on mature shoots (Table 3 and S3). Conversely, degraining increased the grain number and yield of green shoots, which developed late and thereby escaped the degraining treatment, by around 21%. There was no significant effect of degraining on MGW of mature shoots or green shoots when measured separately, although degraining did reduce the overall (total shoot) MGW to a small extent as a result of the larger number of immature grains in the sample.

Additional N fertilizer at anthesis had no significant overall influence (averaged over variety and degraining treatments) on the yield or grain numbers of mature shoots or the combined mature plus green shoots, although it did reduce their MGW by 2–3 mg. By contrast, the additional fertilizer increased the number and hence yield of immature grains, without affecting their MGW (Table 3).

Straw biomass of mature shoots did not differ between varieties and was not affected by anthesis fertilizer application. It was increased by 24% ($P = 0.011$) by degraining. The straw biomass of green shoots, on the other hand, did differ between varieties and was increased by anthesis N fertilizer as well as degraining.

There were significant interactions between variety and degraining treatments on MGW (Table S3 and S4); MGW tended to be increased by degraining in mature and green shoots of Optic and Westminster but remained unchanged or was reduced in other varieties. There were no, or only weak, interactions between other treatment factors.

3.3.2. Post anthesis N uptake

Post anthesis N uptake was calculated for shoots that were mature at harvest and separately for all shoots including mature and green shoots. In each case analysis of variance found no significant effect of variety on PANU, but a significant effect of anthesis N fertilizer application (Table S5). Degraining reduced ($P = 0.016$) post-anthesis N accumulation by mature shoots, but there was no effect of degraining on PANU when all shoots were included. There were no interactions between any of the treatment factors (Table S5). Fig S1 shows the effects of anthesis N fertilizer and degraining on PANU averaged across the varieties. There was an increase in PANU of all shoots with N application at anthesis averaging 0.52 and 0.61 kg per kg of N applied for intact and degrained plants respectively (an apparent fertilizer recovery of 52% and 61%). Importantly, the lack of a significant interaction between degraining and anthesis N application shows that the response of PANU to additional N fertilizer was not significantly influenced by degraining.

The amount of N available for allocation to the grain was estimated from the amount in the crop at anthesis that could potentially be

Table 3
Main effects of variety and anthesis treatments (additional N fertilizer and partial degraining) on yield and yield components of spring barley in 2014.

		Total				Mature shoots				Green shoots			
		Yield, t ha^{-1}	Grains m^{-2}	MGW, mg	Straw, t ha^{-1}	Yield, t ha^{-1}	Grains m^{-2}	MGW, mg	Straw, t ha^{-1}	Yield, t ha^{-1}	Grains m^{-2}	MGW, mg	Straw, t ha^{-1}
Mean	Aramir	4.69	12319	37.9	9.52	4.33	10834	39.9	8.55	0.37	1485	24.5	0.97
Var	Carlsberg	5.39	13484	40.0	9.82	4.56	10674	42.7	7.93	0.83	2810	29.0	1.89
	Kenia	5.23	14263	36.7	9.68	4.28	10925	39.0	7.73	0.95	3338	28.4	1.95
	Optic	6.14	14167	43.5	8.98	5.68	12803	44.6	8.00	0.46	1364	33.7	0.98
	Westminster	5.95	14322	41.7	9.39	4.64	10426	44.7	7.16	1.31	3896	33.3	2.23
	Zephyr	4.89	12370	39.3	9.13	4.25	10192	41.6	7.53	0.64	2179	29.2	1.60
Mean	0	5.38	13015	41.2	9.59	4.79	11119	43.0	8.23	0.59	1896	30.1	1.36
Anthesis N	40	5.42	13610	39.8	9.43	4.60	10889	42.3	7.75	0.82	2721	29.7	1.68
	80	5.35	13838	38.5	9.25	4.49	10919	40.9	7.47	0.86	2919	29.2	1.78
Mean	Degrained	4.23	10829	39.2	11.15	3.40	8065	42.1	9.04	0.83	2764	29.7	2.11
Degraining	Control	6.54	16146	40.5	7.70	5.85	13886	42.1	6.59	0.69	2260	29.7	1.11
P value	Var	< 0.001	0.013	< 0.001	0.606	< 0.001	0.014	< 0.001	0.074	0.002	0.006	< 0.001	0.016
	Anthesis N	0.926	0.204	< 0.001	0.517	0.234	0.825	< 0.001	0.059	0.015	0.004	0.575	0.037
	Degraining	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.934	< 0.001	0.017	0.008	0.882	< 0.001
LSD (5%)	Var	0.473	1369	1.79	ns	0.591	1380	1.58	ns	0.418	1351	1.47	0.791
	Anthesis N	ns	ns	0.71	ns	ns	ns	0.62	ns	0.195	605	ns	0.328

remobilized and the amount of additional fertilizer applied at anthesis that can be captured by the crop. Plotting the N content per grain against the available N per unit grain number revealed a non-linear relationship, with N content approaching saturation at high N availability (Fig. 6). The greatest N availability per grain was generated through the combination of degraining and application of 80 kg N ha^{-1}. The N content per grain of mature grains in this treatment combination was taken to be the maximum N content that could be achieved. Averaged across varieties the N content at near saturation was 0.96 mg N per grain at a concentration of 2.31%.

The unsatisfied grain N demand was calculated using measured values of the N content per grain of the variety at near saturation, the grain number m^{-2} and the amount of N in the crop at anthesis available for retranslocation to grains. Degraining and variety treatments generated a wide range of unsatisfied grain N demand from around zero to 100 kg N ha^{-1} (Fig. 7). When the unsatisfied grain N demand for individual varieties and degraining treatments (degrained and intact controls) were plotted against PANU of all shoots no significant relationship was found. Model II regression by groups showed that there was no significant difference in the slopes of relationships for different anthesis N-fertilizer levels, but that the constants (elevations of the lines) differed ($P < 0.015$) (Table 4). The 95% confidence intervals indicated that the slopes of the relationships did not differ significantly from zero (Table 4) and in each case less than ~5% of the variation in PANU was explained by the unsatisfied grain N demand ($R^2 \leq 0.051$; Fig. 7).

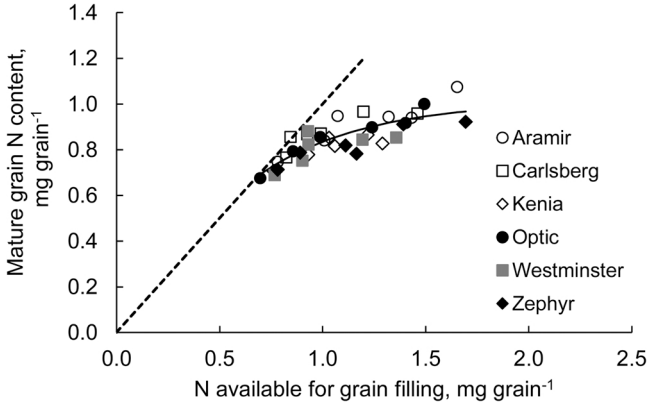

Fig. 6. Relationship between grain N content of mature shoots at harvest and the amount of N potentially available for grain filling. Points are the means of 4 replicates for each combination of variety, anthesis N fertilizer and degraining treatments in 2014. Solid line fitted by linear plus exponential model. Broken line shows 1:1 relationship.

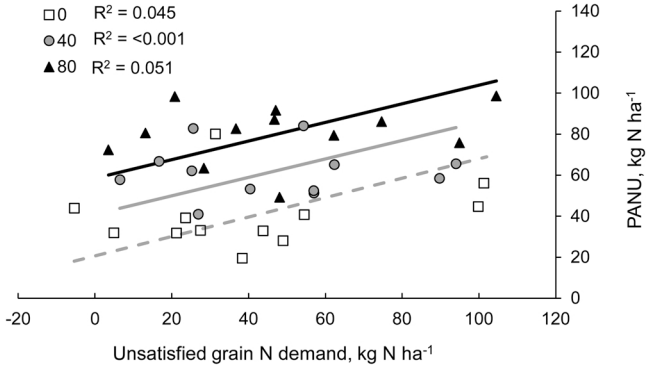

Fig. 7. Relationship between grain N demand that cannot be satisfied by remobilization of N from vegetative tissue and PANU. Each point is the mean for an individual variety x degraining treatment combination at a given anthesis N-fertilizer level (0, 40 and 80 kg N ha^{-1}). Lines fitted by model II regression; slopes not significantly different to zero ($P > 0.05$).

Table 4
Model II regression analysis by groups of relationship between PANU (response variable) and unsatisfied grain N demand (explanatory variable). Groups were anthesis N fertilizer level. P values are for comparisons of slopes and constants. Within a row, constants followed by a different letter are significantly different in pairwise comparisons at $P < 0.05$. CI shows the lower and upper limits to the 95% confidence interval for slopes.

		Anthesis N fertilizer, kg ha^{-1}						
		0		40		80		P value
Constant		20.74	a	40.90	b	58.55	c	< 0.001
Slope		0.48		0.45		0.45		0.990
CI (95%)	lower	-1.049		-0.731		-0.844		
	upper	1.027		0.760		0.762		

3.3.3. N partitioning

The effects of degraining and anthesis fertilizer N application on the partitioning of N between grain and straw at harvest are shown in Fig. 8. There were significant main effects of variety, anthesis N and degraining

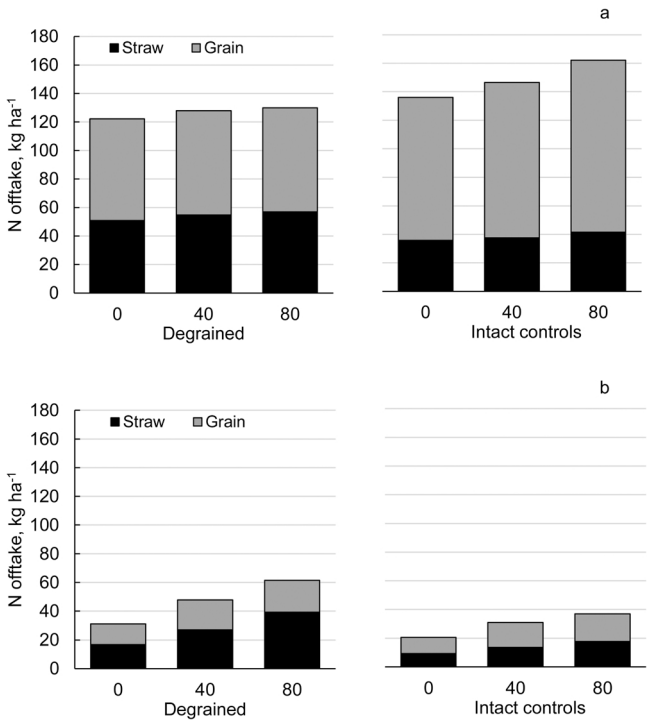

Fig. 8. Effects of degraining and additional N fertilizer at anthesis (0, 40 or 80 kg N ha^{-1}) on N offtake in grain and straw by a) mature shoots and b) green immature shoots at harvest. Values are means over different varieties from anova.

on N offtake by the grain of mature shoots, but only degraining significantly influenced the N offtake of straw (Table S6). There was also a significant interaction (P = 0.033) between anthesis N treatment and degraining on grain N offtake. Thus, degraining reduced N offtake by grain whilst increasing straw N offtake relative to controls (Fig. 8). Anthesis N application increased N offtake by both grain and straw, but the response of grain to the anthesis N was reduced by degraining.

The total N offtake by green shoots was small compared to that by mature shoots. In intact controls it ranged from 20 to 37 kg ha^{-1} for green shoots compared to 136–162 kg ha^{-1} for mature shoots. In degrained plants N offtakes were 31–62 kg ha^{-1} and 122–130 kg ha^{-1} respectively. Degraining increased N offtake by the grain of green shoots by 20% (P = 0.014) when averaged over variety and anthesis N treatments, which contrasts with its effects on the grain of mature shoots. Degraining doubled the N offtake of the straw of green shoots (P < 0.001). Fertilizer N application at anthesis increased N offtake by both the grain (P = 0.003) and straw (P < 0.001) of green shoots. The effect on grains was not influenced by degraining, but for straw the increase was significantly greater in degrained plants than intact controls (anthesis N x degraining interaction P = 0.004).

3.3.4. Relationship between PANU and SMN at anthesis

Data from different years and pre-anthesis treatments were combined to investigate the relationship between PANU and soil mineral N at anthesis without additional N fertilizer application or degraining (Fig. 9). PANU varied widely between site-years and there was no consistent relationship with SMN measured at anthesis. Thus, PANU was considerably lower in 2012 than 2014 in spite of a much larger SMN. The only site where there appeared to be a positive relationship between PANU and SMN at anthesis was Boghall farm in 2011 (Fig. 9).

4. Discussion

Using treatments to vary grain number and fertilizer N application at

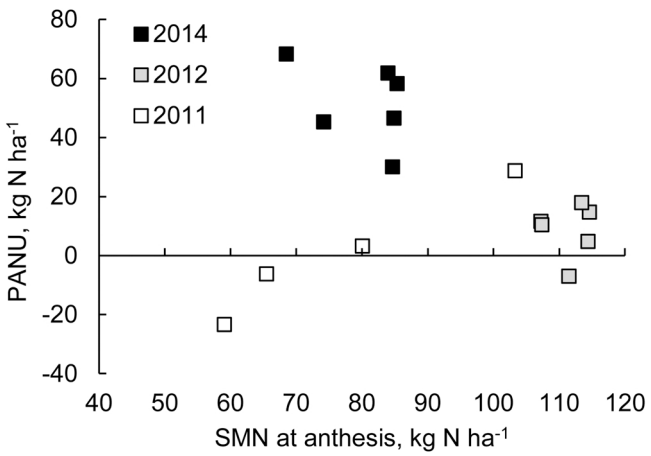

Fig. 9. Scatter plot of PANU in relation to SMN at anthesis for experiments in 2011, 2012 and 2014. Values are means of 4 replicate plots for control plants (non-degrained and given no additional anthesis N fertilizer) of individual varieties (all years) grown at contrasting N fertilizer regimes (2011 and 2012).

anthesis we show that PANU of spring barley may be controlled by both grain N demand and soil N availability, but that in field-grown crops the poise of this control (the major limitation) lies with N availability. The results also show that the response of PANU to variation in grain N demand and N availability was unaffected by pre-anthesis fertilizer regime and thus canopy N status at anthesis and the amount of N available for remobilization to the grain. Evidence to support these conclusions was found in seasons of widely contrasting soil N dynamics. We first discuss the seasonal variation in soil mineral N (SMN) content and crop N uptake before considering the control of PANU.

In 2011 there was a substantial increase in SMN following fertilizer N application after which SMN declined as crop N uptake and accumulation increased. The index SNS is the sum of soil mineral N content within the root zone and the above ground crop N content (N_{off}). Changes in SNS over time indicate the extent of any net gain or loss from the combined SMN plus crop N pool which may occur as result of net mineralization or immobilization of N, or losses of N from the system through leaching or volatilization (King et al., 2001). Following fertilizer application SNS greatly exceeded the reference value calculated as the sum of the SMN, measured prior to crop growth and fertilizer application, and the amount of N fertilizer applied. This suggests there was substantial net mineralization of N soon after fertilizer was applied. Similar observations have been reported previously for UK wheat crops, but the extent of the effect varied with site conditions (King et al., 2001). These authors suggested that the most likely cause of the net mineralization was a temporary disruption of N immobilization. The subsequent decline in SNS prior to anthesis implies that loss of N through immobilization, leaching or gaseous emissions contributed, in addition to crop N uptake, to the depletion of SMN. In 2012, all crops even those supplied with fertilizer at the full recommended rate were clearly N deficient. At anthesis the CAI, above-ground biomass, N content and tissue N concentration were all substantially lower than those that would be expected of crops given adequate nutrition and of those observed in 2011. The grain yields were also considerably smaller and, in spite of the low mean grain weight in 2012, grain N concentrations were extremely low in all fertilizer treatments. Estimates of SNS suggest that there were considerable losses of N from the crop-soil system ranging from 77 to 104 kg ha^{-1} for fertilized plots and 34 kg ha^{-1} for non-fertilized crops over the first 80 days after sowing. Although we have no direct evidence, nitrate leaching and denitrification followed by gaseous emissions of N are likely to account for much of the loss given the unusually high rainfall observed throughout the season (Addiscott and Powlson, 1992). Leaching would also be favoured by the application of fertilizer early in crop development in 2012 before significant root growth had occurred.

Against the contrasting seasonal background of pre-anthesis soil N dynamics and crop N uptake, PANU was consistently increased by the application of additional fertilizer at or shortly after anthesis. The lack of interaction between the pre-anthesis fertilizer regime and anthesis treatment in both 2011 and 2012 indicates that the scale of the response was unaffected by the fertilizer regime and canopy N status at the start of grain filling. This provides strong evidence that under UK barley production systems PANU is controlled by N availability in the root-zone over a wide range of crop N nutritional levels. It is consistent with the agronomic practice of applying fertilizer at flowering of cereals to increase N uptake and grain protein concentration (Gooding et al., 2007; Xue et al., 2016). Our results also indicate that PANU was less responsive to reductions in grain N demand and that site or seasonal factors may determine whether a response occurs. Thus, PANU was reduced by partial degraining in 2012, but was not significantly altered in 2011 and 2014.

As grain growth is the dominant sink for N after flowering it might be expected that reducing grain number by 30–50%, as achieved through partial degraining in the current experiments, would signal a reduction in grain N demand and a down regulation of PANU. Indeed, spikelet removal has been shown to reduce PANU in wheat (Mi et al., 2000; Deng et al., 2019) and that the scale of the response is dependent on the grain sink capacity prior to manipulation (Mi et al., 2000). The principle of feedback regulation of N uptake by plant demand is well established (Imsande and Touraine, 1994; Devienne-Barret et al., 2000; Glass, 2003; Malagoli et al., 2004; Garnett et al., 2013). Regulation may involve both the modulation of specific root membrane transport systems and root system architecture (Glass, 2003; Gojon et al., 2009; Ruffel et al., 2011), however, the signals and molecular mechanisms responsible for the regulation are still uncertain. As plant tissues become replete in N there can be a down regulation of high affinity influx (HATS) and an increase in efflux of nitrate and ammonium (Glass, 2003). Regulation may involve changes in gene expression of transporters and the post-translational modification of proteins (Miller, 2007). Nitrate and reduced N compounds including amino acids and peptides have been implicated in the signalling of plant N satiety to the root system leading to the repression of N uptake by negative feedback (Cooper and Clarkson, 1989; Miller et al., 2007; Okamoto et al., 2016). During grain filling of wheat, expression levels of the high affinity NO_3^- transporter gene *TaNRT2.1* correlated positively with rates of post anthesis NO_3^- uptake by roots and negatively with NO_3^- concentrations in root tissue (Taulemesse et al., 2015). Based on a comparison of wheat genotypes, Taulemesse et al. (2016) postulated that plants of comparable N status (N nutrition index) may differ in their degree of N satiety such that that those with large capacities for luxury N uptake accumulate more N when N availability to the root system is non-limiting and remobilize more N from vegetative tissues when N availability is limiting. Thus, when interpreting the effects of degraining treatments on PANU it is important to consider the overall N satiety of the plant, the role of all sinks in determining that and the interplay between remobilization and PANU in meeting the N demand of the grain and any alternative sinks.

In the current study degraining treatments imposed on varieties contrasting in their grain number resulted in a wide variation in grain N demand that could not be satisfied by remobilization of N from vegetative tissue. Nevertheless, PANU was comparable over this range and the effect of additional N fertilizer on PANU was the same whether the unsatisfied grain N demand was < 20 kg ha^{-1} or > 80 kg ha^{-1}. This implies that grain N demand had little control over PANU in 2014 and that there was a large capacity for accumulating N in sinks other than grain. When grain number was reduced by degraining, grain N content of mature shoots was saturated, and the N content of straw increased relative to intact plants. Degraining also appeared to raise the N content of leaf and stem tissue of mature shoots close to saturation of their storage capacity because anthesis fertilizer only resulted in a small ($P > 0.05$) additional increase in straw N offtake of degrained plants at harvest. However, both degraining and anthesis N fertilizer applications stimulated the growth of late developing tillers in 2014 and these provided a large additional sink for N. The temporal dynamics of tiller production and mortality in spring barley have been found to vary widely between sites and seasons and some late tillering is not uncommon in barley production systems in the temperate climate of the UK and Ireland (Kennedy et al., 2017). Our findings on the importance of alternative sinks in maintaining a demand for PANU by barley following a reduction in grain N demand are supported by comparable work on maize. Prevention of pollination and grain set in maize had little effect on post-silking N uptake because the growth of vegetative organs was stimulated thereby maintaining the overall above-ground N demand (Yang et al., 2016).

As yet unknown site or seasonal factors moderating the effects of late N fertilizer and degraining on post-anthesis tillering may account for why degraining had no effect on PANU in 2014 but reduced it in 2012. A reduction in PANU might occur if degraining satisfies the existing grain and straw N storage capacity but does not stimulate the growth of new sinks. In 2012 and 2011 there was no appreciable effect of degraining or anthesis N application on late tillering. Applying the analysis developed from 2014 data to the experiment in 2012 revealed a weak relationship between the unsatisfied grain N demand and PANU of control and degrained plants in 2012. We must be cautious when interpreting these results because the analysis used estimated values of N content at saturation taken from measurements in 2014. Nevertheless the results, based on two different methods of estimating grain N content at saturation, suggest that grain N demand may have some influence over PANU at levels of satiety that lie below complete saturation of grain N storage capacity. This is consistent with the observed reduction in PANU following degraining even though grain N concentrations were increased to just 1.43% in plants given the standard recommended pre-anthesis N fertilizer regime. This is well below the concentration observed at saturation in 2014 and in commercial crops grown for feed under high N fertilizer regimes. However, only 25% of the variation in PANU was explained by variation in unsatisfied grain N demand and across a large part of this range (50–100 kg N ha^{-1}), PANU was increased by application of additional fertilizer indicating that in the absence of degraining soil N availability was a major limitation to PANU in 2012.

In 2011, PANU of control plants was greater under the full N fertilizer regime rather than those given no fertilizer. Given the large variability associated with estimates of PANU in this experiment the effects were not statistically significant, but the trend towards a greater PANU in fertilized crops is consistent with a greater soil mineral N content at anthesis in the full N treatment. In 2012, PANU of control plants given the full pre-anthesis N regime was significantly lower than those given a low N regime or no fertilizer. The reasons for the lower PANU are unclear. By anthesis SMN contents under all fertilizer regimes had been depleted to comparable levels. However, plant nutritional differences may have resulted in differences in root length and distribution and hence access to SMN during grain filling. Variations in unsatisfied grain N demand may also have contributed to a small extent. Although plants given the full N regime produced larger grain numbers and hence a larger grain sink for N, they also had a greater canopy N content at anthesis and hence more N available for remobilization thereby reducing their estimated unsatisfied grain N demand a little compared to plants given the low N regime. It is important to recognize that in the current study PANU was estimated as the difference in above ground N content between two sampling dates. This is likely to be less accurate than direct estimates made using ^{15}N labelling techniques (Taulemesse et al, 2016) and may account for the apparently negative values of PANU observed in some experiments. However, negative values could also indicate net losses of N from the plant. As such variations in PANU arising from treatments to manipulate grain N demand, including degraining, could conceivably result from effects on N losses from the shoot as well as the regulation of influx by the root system.

The large unused SMN content at harvest (Figs. 2, 3 and Bingham

et al., 2012) does not appear to be the result of a low post-anthesis N demand by the crop. In the absence of degraining and anthesis N fertilizer treatments there was a large excess capacity for N accumulation as grains were unsaturated by N. As such we suggest that increasing grain N demand, or meeting a greater proportion of the demand by improving remobilization efficiency, is unlikely to have a significant effect on the SMN at harvest and hence the risk of nitrate leaching. The large residual SMN content at harvest appears to result from an inability of the root system to access the SMN during grain filling because increasing the amount of readily available N in the form of ammonium nitrate fertilizer increased PANU in all years. There may be a number of factors that contribute to this. The SMN content was calculated from measurements of nitrate and ammonium concentrations in bulk soil sampled from different soil depths. It is recognised that concentrations of N at the root surface are likely to be much lower than those measured in the bulk soil depending on the rate of diffusion and mass flow, which in turn are influenced by soil texture, soil moisture and root length density (Devienne-Barret et al., 2000; Sylvester-Bradley et al., 2001). Inclusion of parameters accounting for the minimum concentration in bulk soil required to drive N uptake at the root surface have proved necessary when modelling nitrate uptake by field-grown crops (Devienne-Barret et al., 2000; Malagoli et al., 2004). Root length density declines with depth through the soil profile and in the subsoil may be less than that required for effective capture of the available nitrate (King et al., 2003; Foulkes et al., 2009). High residual N contents at harvest of some crop species such as *Vicia faba* have been attributed to their low root length densities (Kage, 1997). Further, senescence of the root system during grain filling leads to great uncertainty about how much of the root length present is effective in uptake (Robinson et al., 1991; Kage, 1997).

Results of the current study indicated no relationship between SMN at anthesis (measured to 60 cm depth) and PANU across sites and experimental years over a wide range of SMN, yet in each experiment application of additional fertilizer resulted in an increase in PANU. Application of fertilizer to the topsoil where root length density is greatest may conceivably have increased nitrate and ammonium concentrations at the root surface alleviating the limitation on uptake. The results suggest that measurements of SMN in bulk soil are a poor predictor of N availability to the root system and that access of the root system to SMN during grain filling appears to differ between sites and years. Understanding and overcoming the factors that limit N transfer from bulk soil to those parts of the root system that are active in uptake during grain filling would appear to offer greater scope for increasing the efficiency of N uptake and minimising the risk of post-harvest nitrate leaching than changes to grain N demand.

5. Conclusions

Post-anthesis N uptake by spring barley crops grown under UK field conditions is limited mostly by the availability of N in the soil rather than the grain N demand. PANU could be increased by application of N fertilizer at anthesis but was not consistently modified by reductions in grain number. Relationships between unsatisfied grain N demand and PANU were weak accounting for relatively little variation in PANU. In a standard malting barley production system, there appears to be sufficient capacity for accumulating N in the grains, leaf, and stem tissue of mature shoots, and in the development of new sinks, to drive PANU when N is made available to the root system. When treatments were not applied to vary N source-sink relationships, variations in PANU between sites and seasons were poorly related to measurements of SMN in bulk soil at anthesis. Factors that govern transfer of N to those parts of the root system active in uptake during grain filling may be more important in regulating PANU than soil mineral concentrations themselves. Understanding these factors will be important for maximising the efficiency of N uptake and minimising the SMN residue at harvest.

CRediT authorship contribution statement

Ian Bingham: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization, Supervision, Project administration. **Diana Garzon:** Formal analysis, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors do not have permission to share data.

Acknowledgements

We gratefully acknowledge assistance in the field and laboratory from Colin Crawford and the technical staff of the Crop and Soil Systems research group at SRUC. This work was funded by the Scottish Government's RESAS strategic research programme 2011–2016.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2023.108829.

References

Addiscott, T.M., Powlson, D.S., 1992. Partitioning losses of nitrogen fertilizer between leaching and denitrification. J. Agric. Sci. 118, 101–107.
AnonWorldWeatherOnline 2022. https://www.worldweatheronline.com/penicuik-weather-averages/midlothian/gb.aspx.
Barraclough, P.B., Lopez-Bellido, R., Hawkesford, M.J., 2014. Genotypic variation in the uptake, partitioning and remobilization of nitrogen during grain-filling in wheat. Field Crops Res. https://doi.org/10.1016/j.fcr.2013.10.004.
Bingham, I.J., Karley, A.J., White, P.J., Thomas, W.T.B., Russell, J.R., 2012. Analysis of improvements in nitrogen use efficiency associated with 75 years of spring barley breeding. Eur. J. Agron. https://doi.org/10.1016/j.eja.2011.10.003.
Cooper, H.D., Clarkson, D.T., 1989. Cycling of amino-nitrogen and other nutrients between shoots and roots in cereals - a possible mechanism integrating shoot and root in the regulation of nutrient uptake. J. Exp. Bot. 40, 753–762.
Dawson, C.J., Hilton, J., 2011. Fertiliser availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. Food Policy 36 (SUPPL. 1), S14–S22. https://doi.org/10.1016/j.foodpol.2010.11.012.
Deng, A., Zhang, X., Zhang, X., Qian, H., Zgang, Y., Chen, C., Jiang, Y., Zheng, C., Zhang, W., 2019. Impacts of wheat photosynthate allocation on soil N2O emission during post-anthesis period. Biol. Fertil. Soils 55, 643–648.
Devienne-Barret, F., Justes, E., Machet, J.M., Mary, B., 2000. Integrated control of nitrate uptake by crop growth rate and soil nitrate availability under field conditions. Ann. Bot. 86, 95–1005.
European Comission. (2020). European Commission. Delivering the European Green Deal: A Fair and Just Transition to a Clean Economy. https://ec.europa.eu/info/index_en.
FAOSTAT. (2022). Fertilizers by Nutrient. http://www.fao.org/faostat/en/#data/RFN.
Foulkes, M.J., Hawkesford, M.J., Barraclough, P.B., Holdsworth, M.J., Kerr, S., Kightley, S., Shewry, P.R., 2009. Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. Field Crops Res. 114, 329–342.
Gaju, O., Allard, V., Martre, P., Snape, J.W., Heumez, E., LeGouis, J., Moreau, D., Bogard, M., Griffiths, S., Orford, S., Hubbart, S., Foulkes, M.J., 2011. Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. Field Crops Res. 123 (2), 139–152. https://doi.org/10.1016/j.fcr.2011.05.010.
Gaju, O., Allard, V., Martre, P., Le Gouis, J., Moreau, D., Bogard, M., Hubbart, S., Foulkes, M.J., 2014. Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. Field Crops Res. 155, 213–223. https://doi.org/10.1016/j.fcr.2013.09.003.
Gaju, O., DeSilva, J., Carvalho, P., Hawkesford, M.J., Griffiths, S., Greenland, A., Foulkes, M.J., 2016. Leaf photosynthesis and associations with grain yield, biomass and nitrogen-use efficiency in landraces, synthetic-derived lines and cultivars in wheat. Field Crops Res. 193, 1–15. https://doi.org/10.1016/j.fcr.2016.04.018.
Garnett, T., Conn, V., Plett, D., Conn, S., Zanghellini, J., Mackenzie, N., Enju, A., Francis, K., Holtham, L., Roessner, U., Boughton, B., Bacic, A., Shirley, N., Rafalski, A., Dhugga, K., Tester, M., Kaiser, B.N., 2013. The response of the maize nitrate transport system to nitrogen demand and supply across the lifecycle. *N. Phytol.* Vol. 198 (Issue 1), 82–94. https://doi.org/10.1111/nph.12166.

Glass, A.D.M., 2003. Nitrogen use efficiency of crop plants: Physiological constraints upon nitrogen absorption. Crit. Rev. Plant Sci. 22, 453–470.
Gojon, A., Nacry, P., Davidian, J.C., 2009. Root uptake regulation: a central process for NPS homeostasis in plants. Curr. Opin. Plant Biol. 12, 328–338.
Gooding, M.J., Gregory, P.J., Ford, K.E., Ruske, R.E., 2007. Recovery of nitrogen from different sources following applications to winter wheat at and after anthesis. Field Crops Res. 100, 143–154.
Hawkesford, M.J., Griffiths, S., 2019. Exploiting genetic variation in nitrogen use efficiency for cereal crop improvement. Curr. Opin. Plant Biol. 49, 35–42. https://doi.org/10.1016/j.pbi.2019.05.003.
Imsande, J., Touraine, B., 1994. N demand and the regulation of nitrate uptake. Plant Physiol. 105 (1), 3–7. https://doi.org/10.1104/pp.105.1.3.
Kage, H., 1997. Is low rooting density of faba beans a cause of high residual nitrate content of soil at harvest? Plant Soil 190, 47–60.
Kendall, S.L., Fitters, T.F.J., Berry, P., Hoad, S.P., Bingham, I.J., 2021. Updating nitrogen and sulphur fertiliser recommendations for spring barley. Agric. Hortic. Dev. Board (AHDB) Proj. Rep. No. 635, 183.
Kennedy, S.P., Bingham, I.J., Spink, J.H., 2017. Determinants of spring barley yield in a high-yield potential environment. J. Agric. Sci. 155, 60–80.
Kindred, D., Knight, S., Berry, P., Sylvester-Bradley, R., Hatley, D., Morris, N., Hoad, S., White, C., 2012. Establishing best practice for estimation of Soil N Supply. Agric. Hortic. Dev. Board (Former. HGCA) Proj. Rep. No. 490, 213.
King, J., Gay, A., Sylvester-Bradley, R., Bingham, I., Foulkes, J., Gregory, P., Robinson, D., 2003. Modelling cereal root systems for water and nitrogen capture: towards an economic optimum. Ann. Bot. 91, 383–390.
King, J.A., Sylvester-Bradley, R., Rochford, A.D.H., 2001. Availability of nitrogen after fertilizer application to cereals. J. Agric. Sci. 136, 141–151.
Ladha, J.K., Pathak, H., Krupnik, T.J., Six, J., van Kessel, C., 2005. Efficiency of fertilizer nitrogen in cereal production: retrospects and prospects. Adv. Agron. https://doi.org/10.1016/S0065-2113(05)87003-8.
Le Gouis, J., Cormier, F., Foulkes, J., Gouache, D., Hirel, B., Moënne-Loccoz, Y., 2016. Breeding for increased nitrogen-use efficiency: a review for wheat (T. aestivum L.). Plant Breed. 135 (3), 255–278. https://doi.org/10.1111/pbr.12371.
Malagoli, P., Laine´, P., Le Deunff, E., Rossato, L., Ney, B., Ourry, A., 2004. Modelling nitrogen uptake in oilseed ape cv Capitol during a growth cycle using influx kinetics of root nitrate transport systems and field experimental data. Plant Physiol. 134, 388–400.
Matre, P., Porter, J.R., Jamieson, P.D., Triboi, E., 2003. Modelling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. Plant Physiol. 113, 1959–1967.
Met Office 2022. https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages.
Mi, G., Tang, L., Zhang, F., Zhang, J., 2000. Is nitrogen uptake after anthesis in wheat regulated by sink size. Field Crops Res. 68 (3), 183–190. https://doi.org/10.1016/S0378-4290(00)00119-2.
Miller, A.J., Fan, X., Shen, Q., Smith, S.J., 2007. Amino acids and nitrate as signals for the regulation of nitrogen acquisition. J. Exp. Bot. 59, 111–119.
Miralles, D.J., Abeledo, L.G., Prado, S.A., Chenu, K., Serrago, R.A., Savin, R., 2021. Crop Physiology. Case Histories for Major Crops. In: Sadras, V.O., Calderini, D.F. (Eds.), Barley. Academic Press, Elsevier, pp. 164–195.
Okamoto, S., Tabat, R., Matsubauashi, Y., 2016. Long distance peptide signalling essential for nutrient homeostasis in plants. Curr. Opions Plant Biol. 34, 35–40.
Pask, A.J.D., Sylvester-Bradley, R., Jamieson, P.D., Foulkes, M.J., 2012. Quantifying how winter wheat crops accumulate and use nitrogen reserves during growth. Field Crops Res. 126, 104–118. https://doi.org/10.1016/j.fcr.2011.09.021.
Passioura, J.B., 2010. Scaling up: the essence of effective agricultural research. Funct. Plant Biol. 37, 585–591.
Raun, W.R., Johnson, G.V., 1999. Improving nitrogen use efficiency for cereal production. *Agron. J.* https://doi.org/10.2134/agronj1999.00021962009100030001x.
Rees, R.M., Augustin, J., Alberti, G., Ball, B.C., Boeckx, P., Cantarel, A., Castaldi, S., Chirinda, N., Chojnicki, B., Giebels, M., Gordon, H., Grosz, B., Horvath, L., Juszczak, R., Kasimir Klemedtsson, Å., Klemedtsson, L., Medinets, S., Machon, A., Mapanda, F., Wuta, M., 2013. Nitrous oxide emissions from European agriculture - an analysis of variability and drivers of emissions from field experiments. Biogeosciences 10 (4), 2671–2682. https://doi.org/10.5194/bg-10-2671-2013.
Robinson, D., Linehan, D.J., Caul, S., 1991. What limits nitrate uptake from soil. Plant Cell Environ. 14, 77–85.
Rowell, D.L., 1994. Soil Science: Methods and Applications. Taylor & Francis.
Ruffel, S., Krouk, G., Ristova, D., Shasha, D., Birnbaum, K.D., Coruzzi, G.M., 2011. Nitrogen economics of root foraging: Transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. PNAS 108, 18524–18529.
Sieling, K., Kage, H., 2021. Apparent fertilizer N recovery and the relationship between grain yield and grain protein concentration of different winter wheat varieties in a long-term field trial. Eur. J. Agron. 124, 126246.
Sylvester-Bradley, R., Kindred, D.R., 2009. Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. *J. Exp. Bot.* https://doi.org/10.1093/jxb/erp116.
Sylvester-Bradley, R., Stokes, D.T., Scott, R.K., 2001. Dynamics of nitrogen capture without fertilizer: the baseline for fertilizing winter wheat in the UK. J. Agric. Sci. 136, 15–33.
Taulemesse, F., Le Gouis, J., Gouache, D., Gibon, Y., Alllard, V., 2015. Post-flowering nitrate uptake in wheat is controlled by N status at flowering, with a putative major role of root nitrate transporter NRT2.1. PLoS ONE 10 (3), e0120291. https://doi.org/10.1371/journal.pone.0120291.
Taulemesse, F., Le Gouis, J., Gouache, D., Gibon, Y., Alllard, V., 2016. Bread wheat (*Triticum aestivum L.*) grain protein concentration is related to early post-flowering nitrate uptake under putative control of plant satiety level. PLoS ONE 11(2) 0149668, e0149668. https://doi.org/10.1371/journal.pone.
Tottman, D.R., 1987. The decimal code for the growth stages of cereals, with illustrations. Ann. Appl. Biol. 110 (2), 441–454. https://doi.org/10.1111/j.1744-7348.1987.tb03275.x.
Triboi, E., Triboi-Blondell, A.M., 2002. Productivity and grain or seed composition: a new approach to an old problem - invited paper. European Journal of Agronomy 16, 163–186.
Xue, C., Erley, G.S.A., Rucker, S., Kehler, P., Obenauf, U., Muhling, K.H., 2016. Late nitrogen application increased protein concentraion but not baking quality of wheat. J. Plant Nutr. Soil Sci. 179, 591–601.
Yang, L., Guo, S., Chen, Q., Chen, F., Yuan, L., Mi, G., 2016. Use of stable nitroegn isotope to reveal the source-sink regulation of nitrogen uptake and remobilization during grain filling phase in maize. PLoS One 11 (9), e0162201. https://doi.org/10.1371/journal.pone.0162201.