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Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars

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ABSTRACT

Our objective was to investigate the determinants of genetic variation in N accumulation, N partitioning and N remobilization to the grain post-flowering and associations with flag-leaf senescence, grain yield and grain N% in 16 wheat cultivars grown under high N (HN) and low N (LN) conditions in the UK and France. Overall, cultivars ranged in leaf lamina N accumulation at anthesis from 5.32 to 8.03 g N m^{-2} at HN and from 2.69 to 3.62 g N m^{-2} at LN, and for the stem-and leaf-sheath from 5.45 to 7.25 g N m^{-2} at HN and from 2.55 to 3.41 g N m^{-2} at LN ($P < 0.001$). Cultivars ranged in N partitioning index (proportion of above-ground N in the crop component) at anthesis for the leaf lamina from 0.37 to 0.42 at HN and 0.34 to 0.40 at LN; and for the stem-and leaf-sheath from 0.39 to 0.43 at HN and from 0.35 to 0.41 at LN ($P < 0.001$). The amount of leaf lamina N remobilized post-anthesis was negatively associated with the duration of post-anthesis flag-leaf senescence amongst cultivars in all experiments under HN. In general, it was difficult to separate genetic differences in lamina N remobilization from those in lamina N accumulation at anthesis. Genetic variation in grain yield and grain N% (through N dilution effects) appeared to be mainly influenced by pre-anthesis N accumulation rather than post-anthesis N remobilization under high N conditions and under milder N stress (Sutton Bonington LN). Where N stress was increased (Clermont Ferrand LN), there was some evidence that lamina N remobilization was a determinant of genetic variation in grain N% although not of grain yield. Our results suggested that selection for lamina N accumulation at anthesis and lamina N remobilization post-anthesis may have value in breeding programmes aimed at optimizing senescence duration and improving grain yield, N-use efficiency and grain N% of wheat.

1. Introduction

Nitrogen (N) fertilizer represents a significant cost for the grower and may also have environmental impacts through nitrate leaching and N_2O (a greenhouse gas) emissions associated with denitrification by soil bacteria. Breeding of N-efficient cultivars is one approach to reduce N fertilizer inputs while maintaining acceptable yields (Foulkes et al., 2009). N-use efficiency (NUE) can be defined as the grain dry matter (DM) yield (kg DM ha^{-1}) divided by the supply of available N from the soil and fertilizer (kg N ha^{-1}; Moll et al., 1982). N-use efficiency can be divided into two components: (i) N-uptake efficiency (NUpE; crop N uptake per N available) and (ii) N-utilization efficiency (NUtE; grain dry matter yield per crop N uptake). The contribution of NUpE and NUtE to genetic variation in NUE in wheat varies according to previous studies (Le Gouis et al., 2010). However, in an examination of 39 UK varieties, Barraclough et al. (2010) found that NUtE explained more of the variation in grain yield than NUpE at 5 N rates. Similarly Gaju et al. (2011) reported genetic variability in NUE under low N related mainly to differences in NUtE rather than NUpE. Therefore, understanding the physiological basis of variation in NUtE may offer avenues to increase NUE in wheat grown in NW Europe and elsewhere in the world.

Accumulation and redistribution of N are important processes determining grain yield and grain quality (Simpson et al., 1983; Hirel et al., 2007, Gaju et al., 2011). It is widely understood that N accumulated before anthesis provides the major source of grain N. In wheat, around 50–95% of the grain N at harvest comes from

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the remobilization of N stored in shoots and roots before anthesis (Palta and Fillery, 1995; Kichey et al., 2007). The leaves and stems are the most important sources of N for the grain (Critchley, 2001) with the roots and chaff contributing about 10 and 15%, respectively (Dalling, 1985).

N-remobilization efficiency (NRE; the proportion of N in the crop or crop component at anthesis which is not present in the crop or crop component at harvest) depends on the amount of N remobilised to the grain in the post-anthesis period and on the amount of N stored in vegetative parts at anthesis. Zhen-Yuan et al. (1996) found that, in wheat between anthesis and maturity, the leaves had a higher NRE (0.75) than the stems (0.43) and the roots (0.47). Using ^{15}N labelling, Kichey et al. (2007) also reported higher NRE for leaves (0.76) compared to stems (0.73) and chaff (0.73). Pask et al. (2012) reported NRE of winter wheat cultivar Istabraq at optimal N was higher for the leaf lamina (0.76) compared to the leaf sheath (0.61), chaff (0.56) and true stem (0.48), with corresponding values for NRE at low N of 0.73, 0.56, 0.63 and 0.47, respectively. Higher N remobilization has been observed under low N supply compared to high N supply by Barbottin et al. (2005). In addition, N uptake by the roots post-anthesis can contribute between 5 to 50% of grain N (e.g. Van Sanford and MacKown, 1986; de Ruiter and Brooking, 1994) depending on soil N availability as well as environmental conditions. The amount of N remobilized to the grain is also highly dependent on N stored at anthesis. Therefore, management practices such as high N supply and late N applications can increase grain protein content (Gooding and Davies, 1992; Palta and Fillery, 1993; Bly and Woodward, 2003). Genetic variation in NRE of the vegetative tissues has been reported in wheat from 0.52 to 0.92 (e.g. Cox et al., 1986; Van Sanford and Mackown, 1987; Papakosta and Garianas, 1991; Barbottin et al., 2005; Tahir and Nakata, 2005; Kichey et al., 2007; Pask, 2009).

Several investigations have concluded that the genetic control of N remobilization is linked to the regulation of leaf senescence (Sinclair and De Wit, 1975; Masclaux et al., 2001; Uauy et al., 2006). Stay-green, the ability to retain green area during grain filling, has been related to the N supply-demand balance during grain filling in sorghum (Borrell et al., 2001; Van Oosterom et al., 2010). Martre et al. (2003) suggested that N remobilization is not driven by demand for N by the grain but rather by the source supply from the vegetative tissues. Modifying NRE to delay senescence could be advantageous in feed wheat cultivars to favour a longer period of active photosynthesis during grain filling and higher grain yield, whereas in bread-making cultivars it may not be advantageous due to detrimental effects on bread-making quality associated with lower grain protein concentration. Gaju et al. (2011) reported that the onset of post-anthesis senescence amongst 16 wheat cultivars grown in the field in France and the UK was negatively correlated with the crop NRE under low N supply, whereas under high N supply there was no correlation. This result suggested that under N-limiting conditions grain growth of wheat crops may be mainly source limited; under non-N-limiting conditions grain growth is reported to be mainly sink limited (Fischer, 2008).

This investigation was conducted to evaluate the effects of N fertilizer rate and genotype on N partitioning and post-anthesis N remobilization and associations with flag-leaf senescence, grain yield and grain N% of wheat. Previously we reported variation in NUE, crop NRE and senescence parameters for 16 wheat cultivars grown in field experiments at four sites in the UK and France in two years (Gaju et al., 2011). In summary, a significant N × cultivar level interaction was observed for NUE. Overall genetic variability in NUE under LN related mainly to differences in NUtE rather than NUpE. In this paper, we present an analysis of the genetic variation in N accumulation and N partitioning at anthesis (leaf lamina, stem-and-leaf-sheath and ear) and post-anthesis N remobilization from these crop components and relationships with senescence parameters, grain yield and grain N% carried out at two of the four sites in two years in the same set of experiments. The main hypotheses examined were that: (i) N accumulation at anthesis in leaf lamina and stem-and-leaf-sheath is a determinant of genetic variation in post-anthesis N remobilization from these respective plant components, and (ii) leaf-lamina N remobilization is a determinant of genetic variation in flag-leaf senescence and grain N%. The specific objectives of the present paper are to: (i) investigate the physiological basis of genetic variation in N accumulation in plant components (leaf lamina, stem-and-leaf-sheath and ear) at anthesis and N remobilization and responses to N availability and (ii) examine associations between N accumulation in plant components (leaf lamina, stem-and-leaf-sheath and ear) at anthesis and N remobilization and flag-leaf senescence, grain yield and grain N%.

2. Materials and methods

2.1. Experimental sites and treatments

Field experiments were carried out at two sites: Sutton Bonington, UK (52°50′ N, 1°14′ W) and Clermont-Ferrand, France (45°46′ N, 03°09′ E) in 2006/7 and 2007/8. Seven UK cultivars and nine French cultivars were grown at two rates of N fertilizer. The full experimental details were described by Gaju et al. (2011). In summary, each experiment used a split-plot design in which N treatment was randomized on main plots, cultivar was randomized on the sub-plots and each treatment was replicated three times. The high N (HN) fertilizer treatment was intended to replicate commercial practice and was calculated using methods described in Anon (2000) in the UK and 'Méthode du Bilan' in France using measurements of the amount of mineral N in the soil in February. Each N treatment was applied in 2 to 4 splits with the first split applied in March and the second and third splits applied when the stem was extending (growth stage (GS; Zadoks et al., 1974) 32 to GS37), and the fourth split at around anthesis. All N fertilizer was applied as granules of ammonium nitrate and each split was applied on the same calendar date for the 16 cultivars.

At Sutton Bonington (SB) the level of fertilizer N applied under low N (LN) was 0 and 30 kg N ha^{-1} for 2006/7 and 2007/8, respectively, whilst 210 kg N ha^{-1} was applied in both years under HN conditions. At Clermont-Ferrand (CF) 40 and 240 kg N ha^{-1} of fertilizer N was applied under LN and HN, respectively, in both years. The soil type at SB was clay loam in 2006/7 and sandy loam in 2007/8 whilst at CF it was clay in 2006/7 and clay loam in 2007/8. The cultivars within an experiment were sown at the same seed rate, and across experiments different seed rates were used to establish a target of 200 plants m^{-2} in the spring and ranged from 250 to 350 seeds m^{-2}. All other crop inputs including pest, weed and disease control, and potassium, phosphate and sulphur fertilizers were applied at levels to prevent non-N nutrients or pests, weeds and diseases from limiting yield.

2.2. Crop measurements

Crop growth was assessed in all sub-plots at anthesis and at ripeness maturity from a defined area of 0.64 m^2 (SB) and 0.51 m^2 (CF) by cutting the stems at soil level. All cultivars were sampled on the date of reaching the stage at anthesis but on the same calendar date at ripeness maturity. Following sampling, a random 10% subsample (by fresh weight) of plant material was taken on which the following measurements were carried out. The plant was separated into lamina, stem-and-leaf-sheath and ear (grain and chaff at harvest) and the dry weight was recorded after drying at 80 °C for 48 h. The concentration of the N in the crop components was measured using the Dumas method (Dumas, 1831). Nitrogen Nutrition Index

(NNI) was estimated according to the ratio of the actual above-ground crop N% at anthesis and the critical N%, where the latter was estimated according to the 'critical dilution curve' described for wheat by Justes et al. (1994). At harvest, the sub-plots were machine-harvested to determine the grain yield from an individual sub-plot area of at least 5 m^2. Thousand grain weight was measured on a representative seed sample of 20 g. The seed was counted on a Numigral seed counter (Villeneuve La Garenne, France) at CF and a Contador seed counter (Pfeuffer, Germany) at SB and the weight corrected to 0% moisture. The number of grains per square metre was calculated from the grain weight and combine grain yield. The data were used to determine the above-ground N uptake at anthesis (AGN_A) and at harvest (AGN_H) and the N uptake in the respective crop components at these stages.

N partitioning index (NPI) at anthesis is the proportion of above-ground N at anthesis in the crop component at anthesis. Post-anthesis N remobilization (NR; kg N ha^{-1}) is the amount of N in the crop component at anthesis which is not recovered in the crop component at harvest. Post-anthesis N remobilization efficiency (NRE) is the proportion of N in the crop component at anthesis which is not present in the crop component at harvest (Eq. (1)).

$$\mathrm{NRE} = \frac{N_A - N_H}{N_A} \quad (1)$$

where NRE is the N remobilization efficiency of the crop component, N_A is the amount of N in the crop component at anthesis (kg N ha^{-1}) and N_H is the amount of N in the crop component at harvest (kg N ha^{-1}).

2.3. Soil mineral N sampling

Soil mineral N (0–90 cm) was assessed immediately before sowing and in early February to quantify soil N supply. Samples taken in early February were from Rialto plots only in three replicates of HN conditions. Six cores were used per plot at each soil depth of 0–30, 30–60 and 60–90 cm and all were bulked to give three samples per plot for each soil horizon. Samples were analyzed for soil mineral N (NO_3^- and NH_4^+) as described by Gaju et al. (2011).

Before sowing, soil mineral N at SB was 95.4 kg N ha^{-1} in 2006 and 38.6 kg N ha^{-1} in 2007, and at CF it was 82.0 and 83.0 kg N ha^{-1}, respectively. In early February, soil mineral N at SB was 96.2 kg N ha^{-1} in 2006 and 35.1 kg N ha^{-1} in 2007 and at CF it was 73.4 and 62.1 kg N ha^{-1}, respectively.

2.4. Senescence parameters

Senescence patterns of the flag leaf (for five tagged main shoots at CF in 2006/7 and 2007/8 or of flag leaves in the whole canopy at SB in 2007/8) were assessed visually by recording the percentage green area senesced using a standard diagnostic key based on a scale of 0–10 (100% senesced), as described by Gaju et al. (2011). The same diagnostic key was used at all sites and at each site one operator assessed senescence scores throughout a given season. Assessment was carried out twice weekly after anthesis until full flag-leaf senescence. The data were then fitted against thermal time from anthesis (GS61; base temperature of 0 °C) using a modified version of an equation with five parameters consisting of a monomolecular and a logistic function (Génard et al., 1999). The onset of post-anthesis senescence (SEN_{ONSET}; °Cd) was defined as the onset of the rapid phase of senescence; the rate of post-anthesis senescence was defined as the rate of the rapid phase of senescence (SEN_{RATE}; $°Cd^{-1}$); and the end of post-anthesis senescence (SEN_{END}; °Cd) was defined as the end of the rapid phase of senescence.

2.5. Statistical analysis

Analysis of variance (ANOVA) procedures for a split-plot design were used to analyze N treatments and cultivar effects and test their interaction in individual site-seasons using Genstat version 15.1 (www.genstat.com; VSN International Ltd, Hemel Hempsted, UK), where replicates were regarded as random effects and cultivar as fixed effects. A cross-site-season ANOVA was applied to analyze N treatments and cultivar effects across experiments and the interaction with site-season, assuming N treatments and cultivars were fixed effects and replicates and site-seasons were random effects. Pearson's correlation coefficient and linear regressions using Model II (standard major axis regression; Warton et al., 2006) were calculated to quantify associations between traits using Genstat version 15.1.

3. Results

3.1. N accumulation in crop components at anthesis

At anthesis, in response to N fertilizer, leaf lamina N overall increased from 3.21 g N m^{-2} under LN to 6.89 g N m^{-2} under HN conditions ($P<0.01$; Table 1). The increase in lamina N in response to N fertilizer ranged amongst the cultivars from 2.74 g N m^{-2} (+49%; Récital) to 4.79 g N m^{-2} (+63%; Rialto; $P<0.001$). Stem (true stem plus leaf sheath) N increased in response to N fertilizer from 3.05 g N m^{-2} under LN to 6.36 g N m^{-2} under HN ($P<0.001$), with increases ranging amongst cultivars from 2.37 (+44%; Récital) to 4.07 (+58%; Robigus) g N m^{-2} ($P<0.01$). The site × N × cultivar interaction for leaf N was not significant,. However, for stem N the site × N × cultivar interaction was significant ($P<0.05$) indicating responses of cultivars to N supply depended on site. N in the ear at anthesis increased from 2.02 g N m^{-2} under LN to 2.97 g N m^{-2} under HN; the N × cultivar interaction was not statistically significant.

3.2. N partitioning at anthesis

In these experiments we used the N Nutrition Index (NNI) at anthesis as an indicator of the crop N stress incurred by the cultivars in the N treatments at the two sites (Justes et al., 1994). Averaging across cultivars, with increasing N supply the NNI increased from 0.45 (LN) to 0.81 (HN) at CF and from 0.54 (LN) to 0.99 (HN) at SB, respectively. There was no evidence for a relationship between NNI and N partitioning index at anthesis across site × N treatment combinations for the lamina or the stem, but the ear N index showed a negative linear relationship with NNI at anthesis ($r^2 = 0.68$, $P<0.001$) (Fig. 1). For the ear N partitioning index, for the relationship amongst cultivars within each site × N treatment combination, the linear regression on NNI was, however, only close to statistical significance at CF in the LN treatment ($P = 0.09$).

The leaf-lamina N partitioning index decreased overall with N deficiency from 0.42 (HN) to 0.38 (LN) ($P<0.01$; Fig. 1; Supplementary Table S1); the decrease in lamina NPI at LN was relativey greater at CF than SB and the site × N treatment interaction was significant. Averaging across sites and N treatments, lamina NPI ranged amongst cultivars from 0.37 (Récital) to 0.42 (Renan); and the N × cultivar interaction was significant. Stem NPI overall decreased with N deficiency from 0.39 (HN) to 0.37 (LN) ($P<0.001$). This overall effect related to a decrease in stem NPI at SB under LN but no difference in stem NPI between LN and HN at CF; and there was a site × N interaction ($P<0.01$). Averaging across sites and N treatments, stem NPI ranged amongst cultivars from 0.36 (Alchemy, Robigus, Rialto, Beaver and CF9107) to 0.41 (CF99102

Table 1
Leaf lamina (Lam N_A), stem (Stem N_A, true stem plus leaf sheath) and ear N (Ear N_A) at anthesis for 16 bread wheat cultivars grown at Clermont-Ferrand (CF), France, and Sutton Bonington (SB), UK, under low N (LN) and high N (HN) conditions. Values represent means across the 2006/7 and 2007/8 growing seasons. SED, standard error of the difference of the means; df, degree of freedom; ns, not significant; and Cv, cultivar.

Cultivars	Lam N_A (g N m^{-2})						Stem N_A (g N m^{-2})						Ear N_A (g N m^{-2})					
	CF		SB		Mean		CF		SB		Mean		CF		SB		Mean	
	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN
Alchemy	1.76	4.86	4.94	9.21	3.35	7.04	1.77	4.05	4.11	8.06	2.94	6.06	1.52	2.53	2.49	3.72	2.01	3.13
Arche	2.04	4.61	4.45	9.56	3.25	7.09	2.27	3.94	4.45	8.92	3.36	6.43	1.6	2.58	2.6	3.93	2.10	3.26
Beaver	1.77	5.08	4.91	10.01	3.34	7.55	1.88	4.58	4.2	8.55	3.04	6.57	1.64	3.01	3.11	3.85	2.38	3.43
CF9107	2.24	4.42	4.21	8.19	3.23	6.31	2.19	3.63	3.36	7.78	2.78	5.71	1.74	2.58	2.32	3.14	2.03	2.86
CF99102	1.96	4.05	4.62	9.46	3.29	6.76	2.29	3.93	4.41	10.07	3.35	7.00	1.46	2.39	2.32	3.45	1.89	2.92
Consort	1.66	4.83	4.56	9.55	3.11	7.19	1.65	4.59	3.97	8.07	2.81	6.33	1.42	2.45	2.55	3.17	1.99	2.81
Paragon	1.75	3.98	4.6	9.2	3.18	6.59	2.18	3.98	4.64	9.15	3.41	6.57	1.30	1.89	2.49	3.15	1.90	2.52
Perfector	1.91	4.86	5.33	9.92	3.62	7.39	1.98	4.18	4.26	9.14	3.12	6.66	1.43	2.52	2.5	3.37	1.97	2.95
Quebon	1.86	4.44	4.66	9.56	3.26	7.00	1.91	3.55	4.49	9.43	3.20	6.49	1.42	2.62	2.83	3.81	2.13	3.22
Récital	2.00	3.77	3.37	6.87	2.69	5.32	2.92	3.45	3.24	7.45	3.08	5.45	1.90	2.54	2.02	2.89	1.96	2.72
Renan	2.40	3.97	4.5	8.64	3.45	6.31	2.04	3.01	3.97	8.6	3.01	5.81	1.56	2.21	2.19	3.2	1.88	2.71
Rialto	1.43	3.94	4.2	11.26	2.82	7.60	1.44	3.32	3.93	9.9	2.69	6.61	1.23	2.2	2.68	4.05	1.96	3.13
Robigus	1.75	4.86	4.96	11.19	3.36	8.03	1.72	3.96	4.29	10.19	3.01	7.08	1.69	2.82	2.66	4.01	2.18	3.42
Savannah	1.96	4.72	4.35	9.83	3.16	7.28	2.63	5.21	3.88	9.29	3.26	7.25	1.74	2.89	2.52	3.46	2.13	3.18
Soissons	2.27	3.66	4.39	8.48	3.33	6.07	2.32	3.36	4.01	8.55	3.17	5.96	1.39	1.87	2.25	3.22	1.82	2.55
Toisondor	1.84	4.7	4.1	8.86	2.97	6.78	1.75	4.21	3.34	8.39	2.55	6.30	1.58	2.47	2.36	3.04	1.97	2.76
Mean	1.91	4.42	4.51	9.36	3.21	6.89	2.06	3.93	4.03	8.85	3.05	6.39	1.54	2.47	2.49	3.47	2.02	2.97
SED																		
N, df 8	0.1990***						0.166***						0.071***					
N × Site, df 8	0.352***						0.194***						0.089^{ns}					
Cv, df 236	0.247***						0.228***						0.115***					
N × Cv, df 236	0.393***						0.354**						0.173^{ns}					
Site × N × Cv, df 236	0.540^{ns}						0.483*						0.245^{ns}					

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

and Paragon; $P<0.001$), and there was a N × cultivar interaction ($P=0.08$).

Ear N partitioning index overall increased with N deficiency from 0.20 (HN) to 0.26 (LN), with a smaller increase evident at CF from 0.23 to 0.28 than at SB from 0.16 to 0.23 ($P<0.01$). Averaging over sites and N treatments, ear NPI ranged amongst cultivars from 0.20 (Soissons) to 0.25 (Beaver; $P<0.001$). The response of ear NPI to N deficiency ranged from +0.03 (CF99102) to +0.09 (Toisondor; $P<0.001$). There was a trend for a relatively greater increase in ear NPI with N deficiency for the later flowering cultivars (linear relationship between N response and anthesis date amongst the 16 cultivars; $r^2=0.25$; $P=0.054$, Supplementary Fig. S1). The site × N × cultivar interaction was statistically significant (Supplementary Table S1).

3.3. Post-anthesis leaf-lamina and stem N remobilization and post-anthesis N uptake

Overall lamina NRE was higher at SB (0.83) than CF (0.72) and higher under LN (0.80) than HN (0.75) conditions (Supplementary Table S2; Fig. 2). Averaging across sites and N treatments, cultivars ranged in lamina NRE from 0.74 (Toisondor) to 0.81 (Rialto; $P<0.001$; Supplementary Table S2). Cultivars responded differently to N deficiency, with changes in lamina NRE ranging from -0.01 (Rialto, Robigus, and Savannah) to +0.16 (Soissons). The responses were associated with anthesis date ($r^2=0.25$, $P<0.01$), with earlier cultivars increasing lamina NRE relatively more than later cultivars in response to N deficiency. This may have reflected that there was an inverse relationship between post-antheis N uptake (PANU) and lamina NRE and that early flowering cultivars decreased PANU relatively more than late flowering cultivars under N deficiency (Gaju et al., 2011, Table 2). There was a significant site × N × cultivar interaction ($P<0.001$), indicating that the responses of cultivars to N deficiency depended on site (Supplementary Table S2).

Overall stem NRE was higher at SB (0.61) than CF (0.50); stem NRE increased with N deficiency at CF (from 0.44 (HN) to 0.55 (LN)), but not at SB (Supplementary Table S2; Fig. 2). Averaging across sites and N treatments, stem NRE ranged amongst cultivars from 0.48 (Renan) to 0.61 (CF99102; $P<0.001$). The N × cultivar interaction was not significant.

More N was remobilised in the post-anthesis period from the lamina than the stem under both HN (5.42 vs 3.56 g N m^{-2}) and LN (2.65 vs 1.82 g N m^{-2}) conditions (Figs. 2 and 3; Supplementary Table S3). For both the lamina and the stem, the absolute amount of N remobilised (NR) decreased with N deficiency ($P<0.001$). Lamina NR ranged overall from 3.16 (Récital) to 4.61 (Robigus) g N m^{-2} ($P<0.001$) and stem NR from 2.17 (Renan) to 3.15 (Savannah) g N m^{-2} ($P<0.001$). For lamina NR, the N × cultivar interaction was significant with the reduction under N deficiency ranging from 38% (Soissons) to 64% (Rialto) (Supplementary Table S3). For stem NR, there was a N × cultivar interaction ($P<0.05$), with the reduction in stem NR under LN ranging from 38% (Paragon) to 60% (Toisondor).

The amount of N remobilized from each crop component together with post-anthesis N uptake is shown in Fig. 2 for the site/N treatment combinations. Post-anthesis N uptake decreased with N deficiency from 1.85 to 1.30 g N m^{-2} at SB and from 8.81 to 3.67 g N m^{-2} at CF ($P<0.01$). Post-anthesis N uptake ranged overall amongst cultivars from 2.87 g N m^{-2} (Consort) to 4.91 g N m^{-2} (Renan) ($P<0.05$; Fig. 4). The N × cultivar interaction and the site × N × cultivar interactions were not significant.

3.4. Relationships between N uptake, grains per m^2 and N remobilization

N uptake at anthesis was positively correlated amongst cultivars with each of lamina NR and stem NR in most experiments. At SB, AGN_A,was positively correlated amongst cultivars with each of lamina NR and stem NR under both LN ($r=0.89$, $P<0.001$ and $r=0.88$, $P<0.001$, respectively) and HN ($r=0.97$, $P<0.001$ and

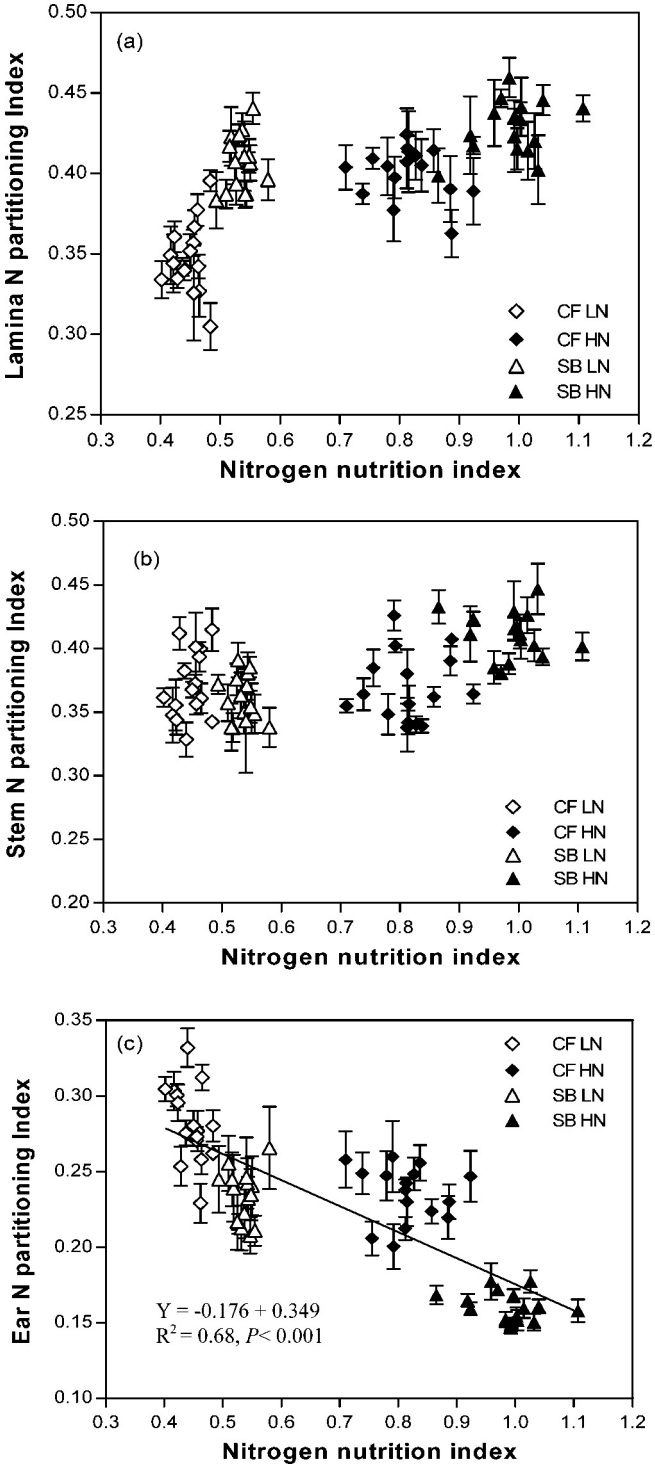

Fig. 1. N partitioning index at anthesis for leaf lamina (a), stem-and-leaf-sheath (b), and ear (c) versus N nutrition index at anthesis for 16 wheat cultivars grown at Clermont-Ferrand (CF), France, under high N (♦; HN) and low N (◊; LN) conditions and at Sutton Bonington (SB), UK, under high N (▲) and low N (△) conditions. Values represent means ± 1 s.e.m. across the 2006/7 and 2007/8 growing seasons.

$r = 0.87$, $P < 0.001$, respectively) conditions. At CF, similar positive associations were found under LN conditions ($r = 0.70$, $P < 0.01$ and $r = 0.69$, $P < 0.01$, respectively). Under HN conditions, AGN_A was only significantly associated with lamina NR ($r = 0.77$, $P < 0.001$). At both sites in each N treatment, there was always a positive correlation between N accumulation in the lamina or stem at anthesis and the subsequent NR from the respective crop component ($P < 0.05$; Table 2).

Post-anthesis N uptake was negatively correlated amongst cultivars with lamina NR ($r = -0.75$, $P < 0.001$) and stem NR ($r = -0.63$, $P < 0.01$) under LN at SB. Similarly negative correlations were found between PANU and lamina NR ($r = -0.79$, $P < 0.001$) and stem NR ($r = -0.80$, $P < 0.001$; Table 2 and Fig. 2) under HN. At CF, negative correlations were again found between PANU and lamina NR ($r = -0.61$, $P < 0.01$) and stem NR ($r = -0.57$, $P < 0.05$) under HN. Under LN conditions, there was a weak negative correlation between PANU and lamina NR ($r = -0.41$, $P = 0.09$) but no correlation between PANU and stem NR.

No relationships amongst cultivars were observed between grain N sink strength, as indicated by grains m^{-2}, and lamina NR or stem NR under LN conditions at either CF or SB. Under HN conditions, grains m^{-2} was positively associated amongst cultivars with lamina NR at SB and CF ($r = 0.64$, $P < 0.01$ and $r = 0.57$, $P < 0.05$, respectively), but there was no association at either site with stem NR.

3.5. Relationships between N remobilization and senescence parameters

Senescence parameters (onset, end and rate of the rapid phase of post-anthesis senescence) were estimated in both years at CF and in 2008 at SB. In each case, there was a linear negative relationship amongst cultivars between lamina NR and SEN_{END} under HN conditions ($P < 0.05$ at SB08 and CF07 and $P = 0.08$ at CF08; Table 3 and Fig. 3; Supplementary Table S4). Under LN conditions, no statistically significant relationship was found. Under HN, stem NR was similarly negatively associated with SEN_{END} at SB08 ($r^2 = 0.39$, $P < 0.01$) and at CF07 ($r^2 = 0.26$, $P < 0.05$). In addition, stem NR was linearly positively associated with SEN_{END} ($r^2 = 0.41$, $P < 0.01$) at CF08 under LN conditions. No consistent associations were found between the other N variables (N accumulation and N partitioning) and the senescence parameters (correlations not shown).

3.6. Relationships between N uptake, N partitioning and N remobilization parameters and grain yield and grain N%

Grain yield at SB was positively associated amongst cultivars with AGN_A ($r = 0.57$, $P < 0.05$) and lamina NR (0.49, $P < 0.05$) and negatively associated with PANU ($r = -0.60$, $P < 0.05$) under LN conditions (Table 2; Fig. 4). Under HN conditions, grain yield was again positively associated with AGN_A ($r = 0.69$. $P < 0.01$) and lamina NR ($r = 0.76$, $P < 0.001$). At CF, grain yield was not associated amongst cultivars with any of the N variables under LN conditions. Under HN conditions, grain yield was positively associated with AGN_A ($r = 0.64$, $P < 0.01$) and lamina NR ($r = 0.74$, $P < 0.001$) and negatively associated with PANU ($r = -0.50$, $P < 0.05$).

Turning to consider grain N%, at SB grain N% was negatively associated amongst cultivars with AGN_A ($r = -0.50$, $P < 0.05$) and lamina NR ($r = -0.43$, $P = 0.08$) and positively associated with PANU ($r = 0.68$, $P < 0.001$) under LN conditions. Under HN conditions, there was a trend for a negative association between grain N% and lamina NR ($r = -0.44$, $P = 0.08$). At CF, grain N% was positively associated amongst cultivars with AGN_A ($r = 0.48$, $P = 0.06$) and lamina NR ($r = 0.58$, $P < 0.05$) under LN conditions. Under HN conditions, grain N% was negatively associated with AGN_A ($r = -0.62$, $P < 0.01$) and lamina NR ($r = -0.67$, $P < 0.01$) and positively associated with PANU ($r = 0.62$, $P < 0.01$).

4. Discussion

The present results allow us to discuss firstly the physiological basis of genetic variation in N accumulation, N partitioning and N

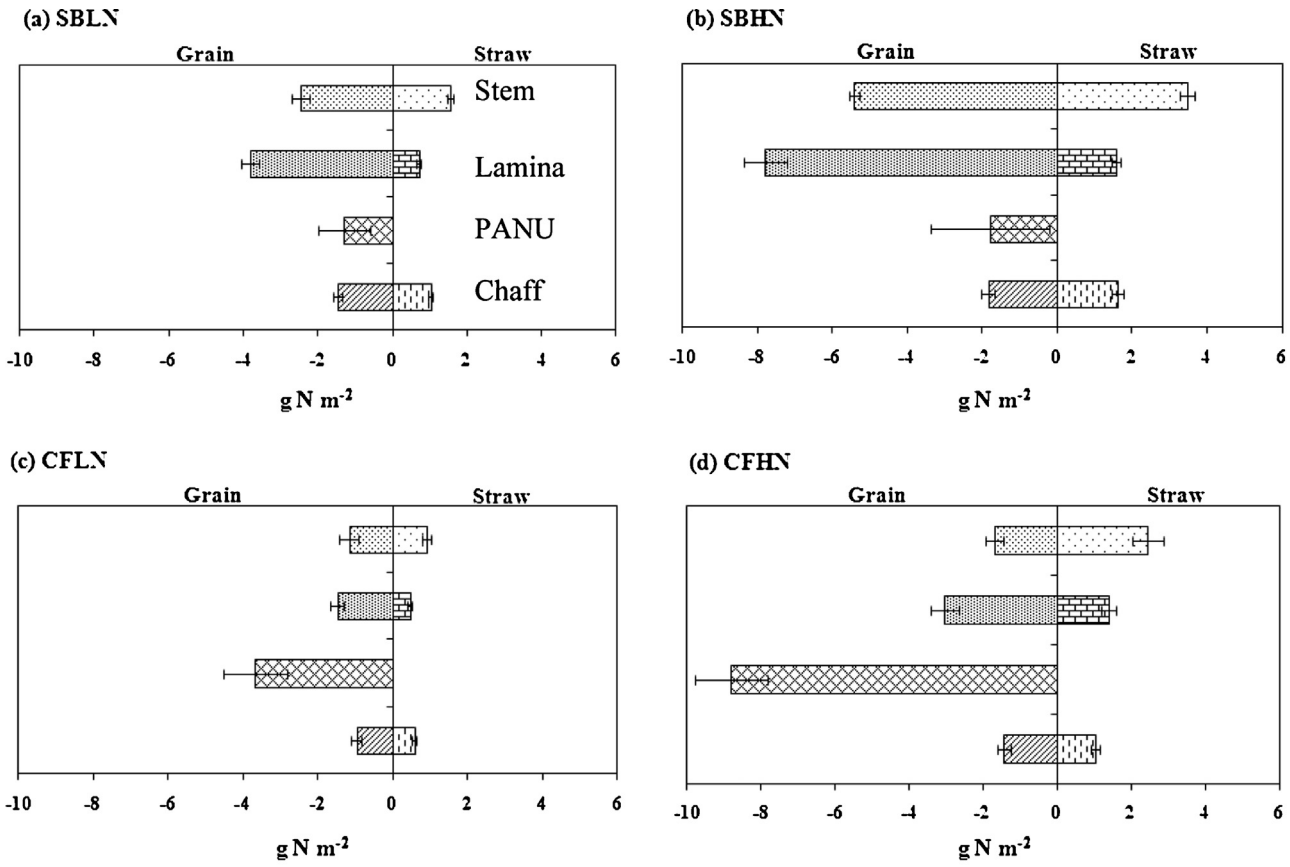

Fig. 2. Negative values on the left hand side of the *y*-axis are the amount of N remobilised post-anthesis to the grain from each crop component (leaf lamina (Lamina), stem-and-leaf sheath (Stem) and chaff) and the post-anthesis N uptake (PANU); positive values on the right hand side of the *y*-axis are the remaining amount of N in each crop component at harvest at Sutton Bonington (SB), UK (a and b) and Clermont Ferrand, France (CF) (c and d) under low N (LN) and high N (HN) conditions. Values represent means across 2006/7 and 2007/8. Errors bars represent s.e.m. across the 2006/7 and 2007/8 growing seasons.

Table 2
Pearson's correlation coefficients amongst 16 wheat cultivars between above-ground N at anthesis (AGN_A, g N m^{-2}), post-anthesis N uptake (PANU, g N m^{-2}), grains per m^2 (GNM2), lamina (Lam N_A, g N m^{-2}) and stem-and-leaf-sheath (Stem N_A, g N m^{-2}) N at anthesis, lamina (Lam NR, g N m^{-2}) and stem-and-leaf-sheath (Stem NR, g N m^{-2}) N remobilization and lamina (Lam NRE, dimensionless) and stem-and-leaf-sheath (Stem NRE, dimensionless) N remobilization efficiency at Clermont-Ferrand (CF), France, and Sutton Bonington (SB), UK, in low N (unshaded) and high N (shaded) conditions, based on means over 2007 and 2008.

	AGN_A	PANU	GY	GN%	GNM2	Lam N_A	Stem N_A	Lam NR	Stem NR	Lam NRE	Stem NRE
SB											
AGN_A	*	−0.80***	0.69**	−0.31	0.56*	0.97***	0.89***	0.97***	0.87***	0.47	0.71**
PANU	−0.72**	*	−0.47†	0.27	−0.52*	−0.75***	−0.81***	−0.79***	−0.80***	−0.59*	−0.64**
GY	0.57*	−0.60*	*	−0.85***	0.86***	0.76***	0.41	0.76***	0.56*	0.46†	0.54*
GN%	−0.50*	0.68**	−0.95***	*	−0.75***	0.41	−0.05	−0.43†	−0.29	−0.43	−0.29
GNM2	0.40	−0.53*	0.84***	−0.87***	*	0.64**	0.33	0.64**	0.42	0.48†	0.48†
Lam N_A	0.91***	−0.70**	0.51*	−0.46	0.32	*	0.78***	0.99***	0.79***	0.42	0.71**
Stem N_A	0.87***	−0.50*	0.30	−0.19	0.23	0.71**	*	0.82***	0.88***	0.53*	0.62*
Lam NR	0.89***	−0.75***	0.49*	−0.44†	0.3	0.98***	0.69**	*	0.83***	0.54*	0.72**
Stem NR	0.88***	−0.63**	0.44†	−0.36	0.32	0.66**	0.91***	0.66**	*	0.58*	0.87***
Lam NRE	0.31	−0.48†	0.23	−0.16	0.10	0.32	0.18	0.51*	0.25	*	0.48†
Stem NRE	0.65**	−0.71**	0.27	−0.38	0.38	0.47†	0.60*	0.50*	0.73**	0.30	*
CF											
AGN_A	*	−0.44†	0.64**	−0.62**	0.59*	0.86***	0.83***	0.77***	0.34	0.50*	−0.07
PANU	−0.06	*	−0.50*	0.62**	−0.55*	−0.48†	−0.38	−0.61**	−0.57*	−0.47	−0.40
GY	−0.28	0.21	*	−0.66**	0.64**	0.56*	0.61**	0.74***	0.35	0.72***	0.02
GN%	0.48†	0.15	−0.60*	*	−0.88***	−0.69**	−0.66**	−0.67**	−0.32	−0.49*	−0.32
GNM2	−0.20	0.09	0.62**	−0.61**	*	0.57*	0.57*	0.57*	0.21	0.40	−0.08
Lam N_A	0.78***	0.02	−0.31	0.64**	−0.41	*	0.72**	0.87***	0.46†	0.58*	0.19
Stem N_A	0.89***	−0.05	−0.21	0.34	−0.09	0.57*	*	0.64**	0.57*	0.44†	0.10
Lam NR	0.70**	−0.41†	−0.33	0.58*	−0.39	0.88***	0.53*	*	0.60*	0.88***	0.38
Stem NR	0.69**	−0.29	−0.06	0.24	−0.07	0.40	0.88***	0.52*	*	0.58*	0.85***
Lam NRE	0.19	−0.72**	−0.15	0.22	−0.31	0.19	0.22	0.58*	0.54*	*	0.49†
Stem NRE	0.15	−0.18	0.32	0.24	0.20	0.04	0.35	0.23	0.66**	0.53*	*

† Significant at the 0.1 probability level.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 3
Pearson's correlation coefficient amongst 16 wheat cultivars between start of senescence (SEN_{ONSET}, °Cd), end of senescence (SEN_{END}, °Cd), rate of senescence (SEN_{RATE}, score °Cd^{-1}), post-anthesis N uptake (PANU, g N m^{-2}), lamina (Lam NR, g N m^{-2}) and stem-and-leaf-sheath (stem NR, g N m^{-2}) post-anthesis N remobilization and lamina (Lam NRE, dimensionless) and stem-and-leaf-sheath (Stem NRE, dimensionless) post-anthesis N remobilization efficiency at Clermont-Ferrand, France in 2006/7 and 2007/8 and Sutton Bonington, UK in 2007/8 under low N (unshaded) and high N (shaded) conditions.

	SEN_{ONSET}	SEN_{END}	SEN_{RATE}	PANU	Lam NR	Stem NR	Lam NRE	Stem NRE
SB08								
SEN_{ONSET}	*	0.24	0.74***	0.24	0.05	−0.09	−0.15	−0.15
SEN_{END}	0.66**	*	−0.36	0.36	−0.55*	−0.62**	−0.39	−0.66**
SEN_{RATE}	0.53*	−0.08	*	0.04	0.27	0.38	0.12	0.31
PANU	−0.39	0.06	−0.35	*	−0.78***	−0.69**	−0.79***	−0.55*
Lam NR	0.43†	0.11	0.31	−0.64**	*	0.72**	0.75***	0.60*
Stem NR	−0.08	−0.14	−0.02	−0.50	0.55*	*	0.70**	0.93***
Lam NRE	0.38	0.05	0.40	−0.79***	0.59*	0.36	*	0.60*
Stem NRE	−0.09	−0.08	−0.20	−0.62*	0.32	0.74***	0.37	*
CF07								
SEN_{ONSET}	*	0.54*	0.03	0.43	−0.21	−0.49	−0.26	−0.51*
SEN_{END}	0.69**	*	−0.75***	0.42	−0.58*	−0.51*	−0.69**	−0.51*
SEN_{RATE}	0.24	−0.52*	*	−0.18	0.41	0.24	0.62*	0.26
PANU	0.19	0.19	−0.04	*	−0.69**	−0.83***	−0.46†	−0.67**
Lam NR	0.28	0.19	0.04	−0.33	*	0.79***	0.86***	0.80***
Stem NR	0.02	0.12	0.12	−0.18	0.69**	*	0.64**	0.88***
Lam NRE	0.06	0.03	0.01	−0.52*	0.83***	0.73**	*	0.76***
Stem NRE	−0.33	−0.12	−0.12	−0.21	0.47	0.86***	0.68**	*
CF08								
SEN_{ONSET}	*	0.69**	−0.17	0.11	−0.61*	−0.04	−0.35	0.13
SEN_{END}	0.67**	*	−0.78**	−0.01	−0.44	−0.25	−0.42	−0.14
SEN_{RATE}	0.11	−0.61*	*	−0.02	0.23	0.31	0.40	0.29
PANU	−0.05	−0.12	0.16	*	−0.47†	−0.50†	−0.76**	−0.64*
Lam NR	0.09	0.30	−0.39	−0.67**	*	0.32	0.73**	0.09
Stem NR	0.64**	0.64**	−0.20	−0.51*	0.51†	*	0.51*	0.92***
Lam NRE	−0.06	0.04	−0.20	−0.81***	0.75**	0.47†	*	0.52*
Stem NRE	0.40	0.44	−0.16	−0.41	0.30	0.77**	0.56*	*

† Significant at the 0.1 probability level.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

remobilization and their responses to N supply in two contrasting environments. Secondly, we will consider the associations with senescence parameters, grain yield and grain N% and the implications of the present results for breeding more N-efficient crops.

4.1. Genetic variation in N accumulation and N partitioning and response to N availability

Above-ground N uptake at anthesis was linearly associated with anthesis date, with later anthesis date favouring N uptake under both HN and LN. Above-ground biomass at anthesis was also linearly positively associated with anthesis date under HN ($R^2 = 0.54$, $P < 0.01$) and LN ($R^2 = 0.42$, $P < 0.01$; data not shown). It is feasible that the larger above-ground biomass with later flowering was associated with larger root biomass favouring N uptake. Alternatively, a longer duration for N accumulation may also have favoured N uptake not associated with any difference in root biomass.

Crop N partitioning at anthesis changed with N deficiency, with proportionally more N in the ear and less in the leaf lamina and stem. Results indicated the ear to be a priority sink for N as N supply became limiting, and suggested that allocation of N to the lamina and stem was decreased before allocation of N to the ear with N deficiency. Pask et al. (2012) also reported ear N partitioning to increase with increasing N deficiency in field experiments for winter wheat grown in the UK. The apparent increase in ear N partitioning with N deficiency as indicated by NNI was linear.

Overall only small cultivar ranges in N partitioning were observed for the leaf lamina (0.37–0.42 HN and 0.34–0.40 LN) and stem (0.39–0.43 HN and 0.35–0.41 LN) in the present study. The reported genetic ranges are slightly greater than those observed by Pask (2009) for four winter wheat cultivars grown in the UK for leaf lamina (0.35–0.36 at high N and 0.32–0.32 at low N) and similar to those observed for stem N partitioning (0.43–0.47 at high N and 0.35–0.41 at low N). Sylvester-Bradley et al. (2010) for 74 cultivars under non-N-limiting conditions reported a slightly extended range for stem N partitioning of 0.36–0.47 to that observed under HN in the present study. Effects of cultivar on N partitioning were not correlated with plant height in the present study or presence/absence of the *Rht-B1 and Rht-D1* semi-dwarfing genes (data not shown). However, there were some correlations with anthesis date. Under LN conditions, at CF cultivars with later anthesis date (range 17 days) tended to partition more N to the ear. This may have been because under LN later anthesis date favoured deeper rooting and an enhanced N uptake capacity during ear growth from booting to anthesis or alternatively a longer time for soil N mineralization with later anthesis enhanced N supply from booting to anthesis. Additionally, under HN, at SB cultivars with later anthesis date (range 15 days) partitioned more N to lamina, presumably associated with more calendar days to anthesis for uptake of N in the lamina.

The genetic ranges in ear N partitioning in the present study were generally slightly wider than the ranges reported by Pask et al. (2012) of 0.18–0.22 under high N and 0.23–0.28 at low N. Under LN conditions, the increased ear N partitioning suggested that the ear may be a prioritized sink for N as mentioned above. The increase in ear N partitioning was associated with an increase in ear N concentration under LN rather than an increase in ear dry mass partitioning. The prioritization of the ear for N partitioning under LN raises the question of whether N concentration in the ear at anthesis is important for floral development (Sinclair and Jamieson, 2006). Present results indicated no association between N concentration in the ear at anthesis and grains per gram of ear dry mass at anthesis amongst cultivars (data not shown) suggesting that assimilate supply to the ear rather than N concentration

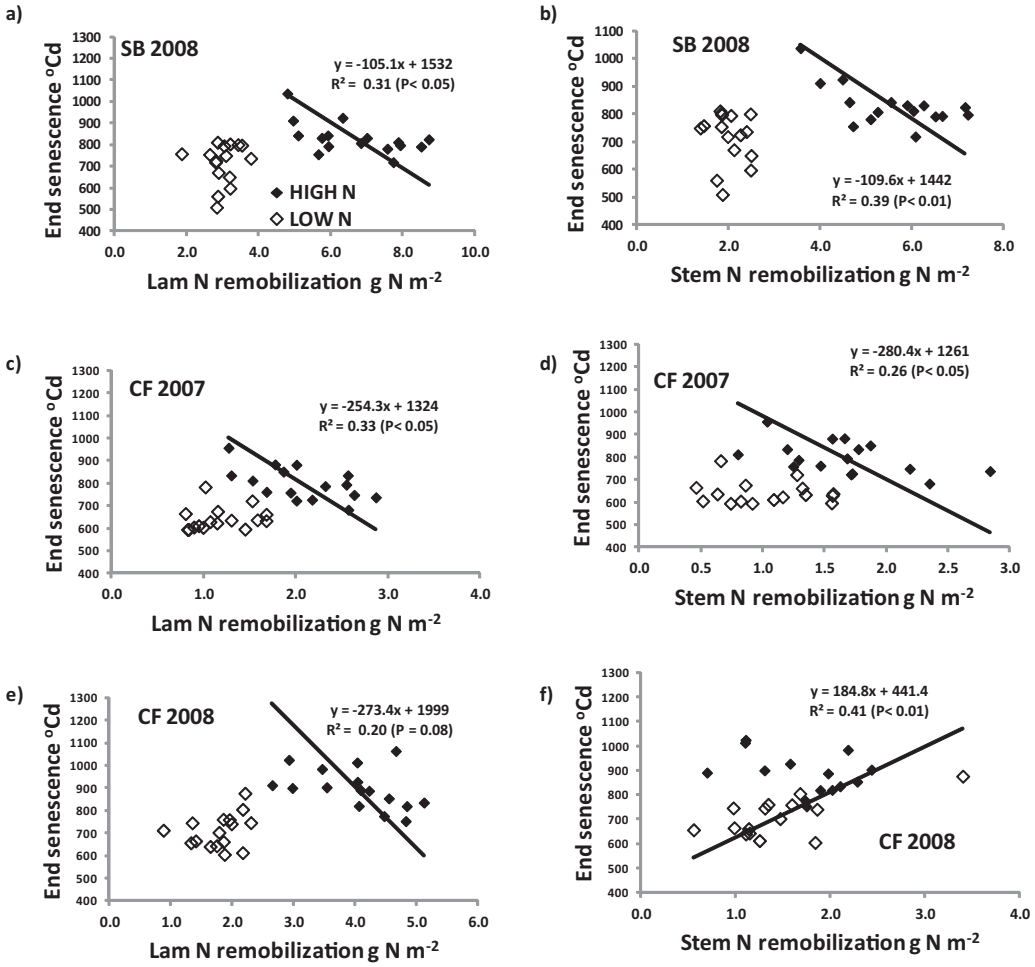

Fig. 3. Relationship between end of post-anthesis senescence of the flag-leaf and amount of N remobilized from the leaf lamina (a,c and e) and the stem and leaf sheath (b, d, and f) for 16 bread wheat cultivars grown at Clermont-Ferrand (CF), France in 2006/7 (c, d) and in 2007/8 (e, f) and at Sutton Bonington (SB), UK, in 2007/8 (a, b) under low (◊) and high (♦) N conditions.

in the ear was the critical determinant of floret survival prior to anthesis in these experiments. Fischer (2008) concluded that there was no evidence for effects of N on grain number apart from those operating via dry matter accumulation; and Gonzalez et al. (2011) demonstrated in wheat that onset of floret death was associated with the beginning of spike growth at the maximum rate consistent with assimilate availability regulating grain number formation.

4.2. Physiological basis of genetic variation in N remobilization efficiency

Present results indicated lamina NRE and stem NRE increased with N deficiency at CF, but not at SB. Kichey et al. (2007) also reported no significant effect of N supply on crop NRE. In the UK, Pask (2009) reported that crop NRE for winter wheat cv. Istabraq was not affected by N treatment in two seasons, whereas in New Zealand as N supply decreased below the 'optimum' N amount leaf-lamina NRE decreased, leaf-sheath NRE was unchanged and true-stem NRE increased. The reason for the absence of an increase in stem or lamina NRE with N limitation at SB in the present study cannot be certain. Under LN conditions, the NNI at SB was slightly higher but not greatly different to that at CF. It is possible that lamina NRE and stem NRE were already high under HN at SB due to a large grain N source supply at anthesis, and that consequently there was limited scope for further increases in NRE under N deficiency. Greater N uptake at anthesis under HN at SB than at CF may also have been associated with relatively more of the above-ground N being allocated as 'reserve' N rather than 'photosynthetic' N or 'structural' N, with 'reserve' N being remobilized post-anthesis with a higher efficiency than the 'structural' or 'photosynthetic N' pools (Pask et al., 2012). Overall cultivars showed greater variation in stem NRE (0.48–0.61) than in lamina NRE (0.74–0.81). Pask (2009) reported a broadly similar range for lamina NRE (0.72–0.78) to the present results, although that author did not find genotypic differences in stem NRE.

4.3. Relationships between N accumulation, N partitioning and N remobilization variables and senescence duration amongst cultivars

Our results indicated that under HN conditions lamina NR was negatively associated with the stay-green trait (end of rapid phase of post-anthesis senescence) amongst cultivars in each experiment. This is in agreement with the 'self-destruction' hypothesis of canopy senescence as N is extracted from the vegetative tissues post-anthesis and remobilized to the grain (Sinclair and De Wit, 1975) and with recent studies in sorghum (Van Oosterom et al., 2010) and the cloning of the NAM-B1 gene from durum wheat (Uauy et al., 2006) linking genetic variation in the pattern of canopy senescence with canopy N remobilization. Although in these experiments senescence duration was not positively associated with grain yield under HN, if grain growth of modern wheat

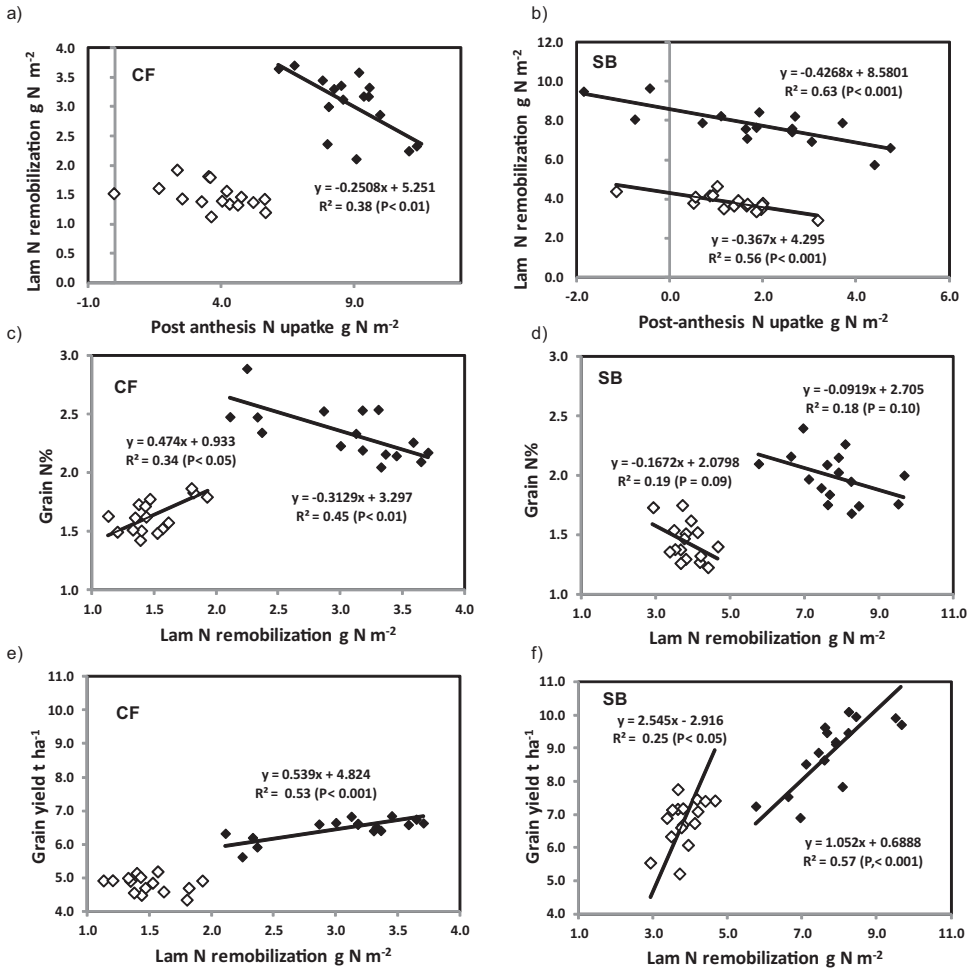

Fig. 4. Linear regressions of post-anthesis leaf-lamina N remobilization on post-anthesis N uptake at (a) Clermont Ferrand and (b) Sutton Bonington; of grain N% on post-anthesis leaf-lamina N remobilization at (c) Clermont Ferrand and (d) Sutton Bonington; and of grain yield (85% DM) on post-anthesis leaf-lamina N remobilization at (e) Clermont Ferrand and (f) Sutton Bonington under low N (◊) and high N (♦) conditions. Values represent means of 2006–7 and 2007–8.

varieties may increasingly become closer to source limitation, or co-limitation by source and sink, under favourable conditions (Acreche and Slafer, 2009), then selection for lower lamina NR may be a beneficial strategy in future breeding programmes, at least in end-use markets for which a high grain starch to protein ratio is desirable, e.g. the feed, distilling or biofuel markets. In the present study, under HN conditions stem NR was also negatively associated with the duration of the flag-leaf green area in two of the three experiments in which senescence was quantified.

In general, in wheat senescence occurs earliest in crops with the least N reserve (Pask, 2009) and in the present study the total duration of senescence was reduced under LN compared to HN in each experiment. Under LN, however, there was no association between lamina NR and senescence duration amongst cultivars. The lack of an association under LN could indicate that the timing of N relocation during the grain filling period rather than the absolute amount of N remobilized over grain filling is the major determinant of lamina senescence duration under LN. Stem NR was associated with the end of senescence in only one out of the three experiments (at CF in 2007/8), and in contrast to HN the association was positive, with higher stem NR associated with the stay-green trait. Foulkes et al. (2009) hypothesized that under N deficiency increased stem NR may delay senescence by buffering lamina N remobilization to the grain during grain filling. Present results were, however, unable to confirm this. Indeed, some caution is required since the positive relationship between stem NR and end of senescence at CF in 2007/8 relied strongly on the performance of one cultivar, Recital, with a long duration of senescence relative to stem NR. Further studies are required characterizing a wider genetic variability in stem NR and the relationship with flag-leaf duration under LN conditions. For example, it is possible there could be a potential trade-off between enhanced stem N partitioning and stem NR and biomass and grain per m^2 at anthesis.

Post-anthesis N uptake by the roots can contribute between 5 to 50% of grain N in wheat (de Ruiter and Brooking, 1994; Kichey et al., 2007) depending on the environmental conditions and soil N availability. In these experiments, the contribution of PANU to grain N was 10.7% (HN) and 14.4% (LN) at SB but much higher at CF (52.2% and 50.3%, respectively). This may have partly reflected the advanced flowering dates at CF compared to SB reducing the capacity for pre-anthesis N uptake relative to grain N sink demand. In all these experiments, the cultivar differences in PANU were not associated with senescence parameters which were more strongly correlated with N remobilization parameters.

Previous evidence in wheat indicates grain N accumulation is likely principally driven by the availability of N from the sources (Triboi and Triboi-Blondel, 2002; Martre et al., 2003), defined as the total non-structural crop N at anthesis. This was demonstrated in experiments by Martre et al. (2003) on four wheat cultivars in which the N source–sink balance was manipulated by removing

the top half of the ear at anthesis. This manipulation resulted in a significant increase in the N concentration of the grain, indicating that the grain N accumulation is regulated by the source and not by the activity of the grain (sink regulated). Borghi et al. (1986) also showed that grain N could be increased by source–sink manipulation, through the removal of 50% of the spikelets at heading which resulted in a 65% reduction in grain yield but a 12–17% increase in grain protein concentration. Present results indicated that lamina NR and stem NR were strongly positively correlated with the N accumulation in these organs at anthesis amongst cultivars under both HN and LN conditions. The corresponding correlations of lamina and stem NR with grains m^{-2} (as an indicator of grain sink size) were statistically significant in only a few cases. Therefore present results support N source size as the principal driver of cultivar differences in N remobilization rather than N grain sink size in wheat.

4.4. Relationships between N accumulation, partitioning and remobilization parameters and grain yield and grain N% amongst cultivars

The relationships amongst cultivars between N uptake, N partitioning and N remobilization parameters and grain yield and grain N% were generally inconsistent across the site/N treatment combinations. This was possibly in part because there was a consistent negative relationship between PANU and lamina NR, which may have confounded relationships between lamina NR and grain yield and grain N%. Some trends, however, were apparent in the results. In three of the four site/N treatment combinations (CF HN, SB HN and SB LN), there was a positive relationship between AGN_A and each of grains m^{-2} and grain yield. In each case, lamina NR was also positively associated with grain yield and negatively associated with grain N% (through dilution effects). The positive association amongst cultivars between lamina NR and grain yield was likely an indirect effect of the positive association between lamina N uptake at anthesis and lamina NR, with the driver for grain yield being the lamina N uptake at anthesis (increasing assimilate supply hence grains m^{-2}) rather than lamina NR per se. In the site/N treatment combination with the highest N stress (as indicated by NNI) at CF under LN conditions, there was no correlation between AGN_A and either grains m^{-2} or grain yield; and, in this case, lamina NR was positively correlated with grain N% amongst the cultivars. Post-anthesis N uptake was as expected generally negatively associated with pre-anthesis N uptake amongst the cultivars (e.g. at CF HN, SB HN and SB LN). This likely explained why PANU was negatively associated with grain yield in each of these site/N treatment combinations, i.e. that pre-anthesis N uptake was the main driver for grain yield variation amongst the cultivars. In general, these results indicated it was difficult to separate genetic variation in lamina N remobilization from that in lamina N accumulation at anthesis. Genetic variation in grain yield and grain N% (through N dilution effects) appeared to be mainly determined by pre-anthesis N accumulation rather than post-anthesis N remobilization under high N conditions and under milder N stress (SB LN). Where N stress was increased (CFLN), there was some evidence that lamina N remobilization was a causal determinant of genetic variation in grain N% although not of grain yield. Since grain yield and grain N% can only be precisely measured in later generations in core breeding programmes, measurements of physiological traits in intermediate generations, offers the possibility of selecting physiologically adapted material before yield and grain quality testing begins. It is suggested that screens for lamina N accumulation at anthesis and lamina NR including molecular markers may have value in breeding programmes aimed at optimizing senescence duration and improving grain yield, NUE and grain N% of wheat.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2013.09.003.

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