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Decorrelating source and sink determinism of nitrogen remobilization during grain filling in wheat

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• *Background and Aims* Nitrogen (N) remobilization is the major source of N for grain filling in wheat, the other being N uptake after anthesis (N_{up}); however, variations in remobilization efficiency are not fully understood. It is hard to tell whether the source or the sink effects predominate, because N in the culm at anthesis (N_{ant}) correlates strongly with both N remobilization (N_{rem}) and grain number (G_n), respectively the main source and the main sink.
• *Methods* A pot experiment was thus designed to assess the relative contributions of the source and sink to N_{rem} regulation. Using two cultivars of winter wheat (*Triticum aestivum*, 'Apache' and 'Autan'), three pre-anthesis and two post-anthesis N fertilization levels were applied in order to vary the N sources, while ear trimming at anthesis reduced sink size.
• *Key Results* Unlike results observed at a scale of m^2, the equation binding N_{ant} to N_{rem} exhibited a negative intercept, challenging the concept of nitrogen remobilization efficiency. Before ear trimming, G_n fitted well to N_{ant}, with a slope dependent on genotype. To obtain a sink variable that was less correlated with N_{ant}, the difference δG_n was calculated between actual grain number and that which could be predicted from culm N before trimming. A multiple regression then predicted N_{rem} ($r^2 = 0{\cdot}95$) from N_{ant}, N_{up} and δG_n, with fitting unbiased by fertilization treatment, trimming or genotype.
• *Conclusions* In untrimmed culms, δG_n had a negligible effect, so that N_{rem} could be fitted to N_{ant} and N_{up} only: grain N filling appeared to be determined by sources only (N_{ant} and N_{up}), not by sink, and the reduction of N_{rem} by N_{up} was quantified. In these 'normal' cases, the regulation of N_{rem} should thus be located within the N sources themselves. In contrast, ear-trimming needs to be considered with caution as it introduced a sink limitation on N_{rem}; moreover one with an important genotype effect.

Key words: *Triticum aestivum*, winter wheat, source/sink, grain filling, nitrogen uptake, grain number, nitrogen harvest index, nitrogen remobilization efficiency, genotype × environment.

INTRODUCTION

Grain protein concentration (GPC) is one of the principal criteria listed in grain specifications for both the processing of wheat flour (*Triticum aestivum*) and the export of grain (Gooding *et al.*, 1994). In a world seeing an ever-growing demand for grain production, increasing grain nitrogen (N) yield is the only way to improve GPC. Thus many genotype and/or management research programs have been undertaken to improve N yield under a range of climatic conditions (Brancourt-Hulmel *et al.*, 2003, 2005). Environmental concerns also require optimized use of the two sources of grain N filling, i.e. (1) the remobilization of nitrogen (N_{rem}) already present in vegetative parts at anthesis (N_{ant}), and (2) post-anthesis nitrogen uptake (N_{up}; see Table 1 for a list of abreviations used in the text).

However, the factors that determine N fluxes during grain filling remain far from clear. According to Martre *et al.* (2003), grain N yield is mostly source-limited, while Barbottin *et al.* (2005) suggested that sink capacity was the main determinant for variations in N_{rem}. In fact, it is difficult to distinguish between sink and source, as they are so strongly correlated that grain number can be accurately modelled using N_{ant} (Abbate *et al.*, 1995; Oscarson, 2000; Demotes-Mainard and Jeuffroy, 2001). Some modellers have even suggested discarding the use of grain number, leading to sink-less models (Sinclair and Jamieson, 2006, 2008). As early as 1979, Martinez-Carrasco and Thorne used surgical ear trimming at around anthesis to disconnect the sink and source, and numerous subsequent papers have reported similar treatments. Both GPC and amount of N per grain always increased following trimming, reaching levels far higher than those observed in control plants, while N yield per ear generally declined, with marked genotype and treatment interactions. According to Ma *et al.* (1996), N yield was only maintained in some cultivars following moderate trimming (25 % of grains removed); however, trimming experiments did not provide clear evidence for a sink limitation of grain N filling. Indeed, several reports have suggested that as well as modifications to the sink, trimming could also induce a fall in N_{rem} or N_{up}, and thus cause a decrease in the source (Mi *et al.*, 2000). Data on the quantitative N balance at the whole-plant level would therefore be welcome in trimming studies, but have seldom been reported.

The simplest way to modify the source/sink ratio during grain N filling may be to increase N_{up} by delaying the last application of fertilizer. This type of crop management has been widely employed in France during the past 10 years; nitrogen application rates are split into three parts so that the

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TABLE 1. *Abbreviations used in the text*

N_{ant}	N amount in vegetative parts, including roots, at anthesis (after ear trimming, if performed)
$N_{ant,i}$	N amount before ear trimming
G_n	Ear grain number measured at the end of grain filling
δG_n	Difference between actual G_n and its predicted value obtained from the genotype-dependant correlation to $N_{ant,i}$
N_{up}	Nitrogen uptake by the culm after anthesis
N_{rem}	Nitrogen remobilization from vegetative parts during grain filling
GPC	Grain protein concentration at the end of grain filling
NRE	Nitrogen remobilization efficiency (ratio of N_{rem} to N_{ant})

last fertilization is delayed to around heading. However, fertilization cannot be delayed indefinitely, because the efficiency of N uptake rapidly declines after anthesis, possibly because of leaf senescence. The improved N yield of 'stay green' cultivars largely arises from their continued N uptake after anthesis, probably because of delayed leaf senescence (Borrell *et al.*, 2001). Generally speaking, it appears that a higher level of N uptake after anthesis leads to delayed N decline in vegetative tissues (Martre *et al.*, 2006). In an opinion paper, Barneix (2007) even suggested N uptake and remobilization might be mutually incompatible due to reciprocal inhibition: N remobilization would only – and irreversibly – start once N uptake had slowed down. This point of view is difficult to sustain at the whole-plant level, as early leaves have already senesced well before anthesis, at a time when N uptake is very high. Instead, it should be noted that N_{rem} is actually a balance between N input and output from vegetative tissues, the former probably increasing in line with N_{up} levels. Regarding the whole period of grain filling, late fertilizer applications have also been shown to reduce the efficiency of N remobilization (Gooding *et al.*, 2007). Conversely, Triboï and Triboï-Blondel (2002) noted that plants with a lower N_{ant}, and hence a lower N_{rem} capacity, have a greater propensity to take up nitrogen after anthesis.

In fact, most of the evidence for antagonism between N_{rem} and N_{up} is based on variations in N concentrations in vegetative parts at the end of the grain filling period. When available, quantitative N balances at the whole-plant level tend to suggest that the losses in N yield due to antagonism between N_{up} and N_{rem} are quite small. This may be due to the fact that under current crop management methods, N_{up} is much lower than N_{rem} during grain filling (Van Sanford and MacKown, 1987); therefore any N_{up} antagonism to N_{rem} would remain moderate and can thus be ignored. This situation is favourable for farmers; however, it could be reversed, because current trends in both plant breeding and crop management are leading to an increase in post-anthesis N uptake. The antagonism between N_{rem} and N_{up} thus needs to be quantified under conditions that lead to higher N_{up}/N_{rem} ratios, because gains in N availability through an increase in N_{up} may be counteracted by a decrease in N_{rem}; in which case, the apparent efficiency of fertilizers would be reduced by increasing the amount of N wasted in straw.

This paper focuses on the regulation of N_{rem} using two wheat cultivars that differ in terms of their grain N filling. A broad range in N_{ant}/N_{up} ratios was obtained by combining three levels of N fertilization before anthesis with two levels afterwards. The specific effect of grain number reduction through ear trimming was also studied. Analyses of variance and multiple correlations were used to explore the regulatory relationships between N_{rem}, N_{up} and grain N filling.

MATERIALS AND METHODS

Culture and treatments

Two modern cultivars ('Apache' and 'Autan') of winter wheat (*Triticum aestivum* L.) were chosen because of their contrasting patterns of N metabolism. In preliminary field trials, 'Apache' always produced more ears per m^2, while grain numbers per ear (main culm and tiller mixed) were similar in the two genotypes. N uptake per m^2 before anthesis was greater in 'Apache', but N uptake after anthesis was higher in 'Autan'. The mean grain weight, GPC and amount of N per grain were higher in 'Autan'.

Seeds were sown on 20 October 2004 in an experimental field at the INRA station in Thiverval-Grignon, France (48°50′N; 1°57′E) at a density of 250 seeds m^{-2}, under either high or no N fertilization (no-N). Highly fertilized plots were sown in a silt loam, a typical Eutrochrept soil (according to soil taxonomy), while no-N plots were sown in the poorest areas in a sandy embankment. On 25 March, plants in the plots had reached the beginning of stem elongation (growth stage = 31, according to Zadoks *et al.*, 1974). No-N plots exhibited a nitrogen nutrition index of 0·4 (NNI; Justes *et al.*, 1994), while the NNI of highly fertilized plots was 0·9. About 560 plants were carefully collected from each of the no-N plots and 280 plants from each of the high-N plots. Their roots were washed extensively before plants were transplanted into perlite-filled pots (two plants per 1·7-L pot) that were then placed outside. The average temperature was 11·5 °C during the next 2 months before anthesis and 17·4 °C during the 2 months after that. During pot culture prior to anthesis, one half of the plants transplanted from the no-N plot received 2·7 mg N per plant on a weekly basis; all other plants received 10·8 mg N per plant. Apart from nitrogen, all plants received the same full fertilization each week, and water when required depending on the weather. Three levels of early fertilization (applied before anthesis) were thus attained: some plants had received low fertilization (low in field, then low in pot), while others had received increasing fertilization (low in field, then high in pot), and others had received high fertilization (high in field, then high in pot).

Ear emergence was recorded for every main culm in order to select 84 pots bearing synchronous plants within each cultivar and early fertilization treatment group. After anthesis, one half of the selected plants received low fertilization, while the other half received high fertilization (1·8 and 7·2 mg N weekly per plant, respectively). Obviously, this latter fertilization could only be absorbed after anthesis; however, because the pots were not washed at anthesis, some of the fertilizer applied before anthesis may have been absorbed afterwards. Thus early and late fertilization could not be regarded as strictly equivalent to N uptake before and after anthesis, respectively. Conversely, N supply and N harvest were balanced over the whole season, suggesting that over this time scale (that of the study) no N leaching occurred from pots.

TABLE 2. *Summary of the different genotypes and treatments (crossing of genotype, fertilization before/after anthesis and ear reduction) used in the study. Treatment identities are used in Fig. 3*

Treatment identity	Genotype	Early fertilization	Late fertilization	Ear reduction
A	'Apache'	Low	Low	Control
B	'Apache'	Low	Low	50 % trimming
C	'Apache'	Low	High	Control
D	'Apache'	Low	High	50 % trimming
E	'Apache'	Increasing	Low	Control
F	'Apache'	Increasing	Low	50 % trimming
G	'Apache'	Increasing	High	Control
H	'Apache'	Increasing	High	50 % trimming
I	'Apache'	High	Low	Control
J	'Apache'	High	Low	50 % trimming
K	'Apache'	High	High	Control
L	'Apache'	High	High	50 % trimming
M	'Autan'	Low	Low	Control
N	'Autan'	Low	Low	50 % trimming
O	'Autan'	Low	High	Control
P	'Autan'	Low	High	50 % trimming
Q	'Autan'	Increasing	Low	Control
R	'Autan'	Increasing	Low	50 % trimming
S	'Autan'	Increasing	High	Control
T	'Autan'	Increasing	High	50 % trimming
U	'Autan'	High	Low	Control
V	'Autan'	High	Low	50 % trimming
W	'Autan'	High	High	Control
X	'Autan'	High	High	50 % trimming

Within 2 d of anthesis, the ears on one half of the plants were trimmed by removing all the odd-numbered spikelets. The experiment as a whole thus consisted 24 treatments with four factors: two genotypes; three early fertilizations; two late fertilizations; and two ear-trimming treatments (Table 2). This provided a fully cross-factor experimental set-up designed to analyse individual effects and interactions. Each treatment was applied to 24 pots, but hereafter only the first and last sampling times are considered, i.e. six pots per treatment, which were harvested as detailed below.

Sampling procedure

Three pots containing two plants each were harvested from each treatment at anthesis (17–26 May, depending on the treatment) and at physiological maturity (around 15 July). The roots and above-ground parts were collected, and within the above-ground parts the main culms were separated from tillers. Roots were recovered quantitatively, but they were usually fragmented and therefore could not be directly attributed to either the main culm or the tillers. Instead, the N ratio of the main culm to tillers was calculated for all above-ground parts of each sample, and used to split the corresponding root nitrogen between the main culm and tillers. The samples were dried for 48 h at 80 °C except for the ears, which were freeze-dried. The grains were then separated from the chaff. The spikelets removed from the trimmed ears were also reserved for further analysis. Tiller number and growth varied considerably within a treatment group. Moreover, under some treatments tillers appeared after anthesis and headed without elongation of their stem. The data concerning tillers should therefore be regarded as being of little significance; hereafter in this paper, only the main culm is considered.

Assessments of N balances

The dry weight of each sample was measured and then finely ground, and N concentrations were subsequently determined using Dumas' combustion method. N uptake (N_{up}) and net remobilization from vegetative organs (N_{rem}) were derived from Ruske *et al.* (2003) except that, unless specifically indicated, these equations also involved root N:

$$N_{up} = (\text{total N in culm at maturity} - \text{total N in culm at anthesis}) \quad (1)$$

$$N_{rem} = (\text{vegetative N at anthesis} - \text{vegetative N at maturity}) \quad (2)$$

Hereafter in this paper N at anthesis (N_{ant}) and grain number (G_n) both refer to the values obtained after ear trimming, in cases where this was done. In some specified cases, estimates prior to trimming ($N_{i,ant}$ and $G_{i,n}$) are also used. The initial grain number ($G_{i,n}$) in trimmed ears could be estimated as being twice the value of G_n recorded after trimming. Trimming also removed some chaff N, which was measured in corresponding samples. Nitrogen before trimming ($N_{i,ant}$) was calculated by correcting N_{ant} for the nitrogen discarded in the spikelets that were removed.

Data analysis

Analyses of variance were performed using Statgraphics Plus (Manugistics, Inc., Rockville, MA) to examine the effects of different genotypes and treatments on various N remobilization parameters. The individual effects of genotype, early fertilization, late fertilization and ear trimming were analysed, as well as their first-level interactions. Statistically significant differences were then determined using the Newman–Keuls test with an overall error rate $\alpha = 0{\cdot}01$.

The contributions of N_{ant}, G_n and N_{up} to N_{rem} were analysed using simple and multiple linear regressions. Slopes and intercepts of the regression lines between genotypes were tested for significance and compared using the specific Statgraphics Plus procedure. Finally, analyses of variance were performed on the residuals of the regressions thus described, in order to detect any bias linked to either genotype, early fertilization, late fertilization or ear trimming.

RESULTS

General features of treatments

The various genotypes and treatments resulted in broad variations in main-culm nitrogen at anthesis (N_{ant}; Table 3). Analysis of variance indicated no significant difference in N_{ant} between genotypes under low, early fertilization (17 ± 3 mg culm^{-1}; mean $\pm$ s.e.), while under increasing or high

TABLE 3. *(a) Nitrogen variables in main culms for the different genotypes and treatments, as listed in Table 2. Anthesis N (N_{ant}) is the amount of N in vegetative parts (including roots) at anthesis (after ear trimming, in cases where this was done); ear grain number (G_n) was obtained at the end of grain filling; N uptake (N_{up}) and remobilization (N_{rem}) were calculated from the differences between data at anthesis and at the end of grain filling. (b) ANOVA was performed by fully crossing the effects of genotype, early fertilization, late fertilization and ear reduction, as well as first-order interactions between the different factors*

(a) N variables

	N_{ant} (mg)	G_n	Grain N (mg)	N_{up} (mg)	N_{rem} (mg)	NRE
Genotype (G)						
'Apache'	30^a	38^b	$1{\cdot}4^a$	26^a	21^b	$0{\cdot}67^b$
'Autan'	26^a	29^a	$1{\cdot}5^b$	28^a	15^a	$0{\cdot}56^a$
Early fertilization (E)						
Low	17^a	24^a	$1{\cdot}4^a$	24^a	10^a	$0{\cdot}57^a$
Increasing	27^b	33^b	$1{\cdot}5^a$	30^a	17^b	$0{\cdot}61^{ab}$
High	42^c	42^c	$1{\cdot}5^a$	27^a	28^c	$0{\cdot}66^b$
Late fertilization (L)						
Low	28^a	34^a	$1{\cdot}3^a$	23^a	19^a	$0{\cdot}64^a$
High	28^a	33^a	$1{\cdot}5^b$	31^b	17^a	$0{\cdot}58^a$
Ear reduction (R)						
Control	30^a	44^b	$1{\cdot}2^a$	30^a	21^b	$0{\cdot}68^a$
50 % trimming	27^a	22^a	$1{\cdot}7^b$	24^a	16^a	$0{\cdot}55^a$

(b) ANOVA

	N_{ant} (mg)	G_n	Grain N (mg)	N_{up} (mg)	N_{rem} (mg)	NRE
G		***	**	ns	***	***
E	***	***	ns	ns	***	*
L	nd	ns	***	*	ns	*
R	ns	***	***		***	***
G × E		***		ns	**	ns
G × L	nd	ns	ns	ns	ns	ns
G × R	ns	ns	**			**
E × L	nd	ns	ns	ns	ns	ns
E × R	ns	***	*	ns		ns
L × R	nd	ns	ns	ns	ns	ns

In (a) different letters indicate a significant difference between means ($P < 0{\cdot}01$; $n = 72$).

In (b) * $P < 0{\cdot}01$; ** $P < 0{\cdot}001$; *** $P < 0{\cdot}0001$; ns denotes non-significant effects ($P > 0{\cdot}05$); nd indicates that the effect of late fertilization did not apply to N_{ant}.

fertilization, N_{ant} was higher in 'Apache' than in 'Autan' (up to 48 ± 5 vs. 39 ± 2 mg culm^{-1}, respectively). Despite the fact that trimming discarded some chaff N (4 ± 1 mg culm^{-1}, averaged over all treatments), N_{ant} was not significantly lower in trimmed plants ($P > 0{\cdot}05$).

Grain number (G_n) ranged from $16{\cdot}3 \pm 0{\cdot}9$ in trimmed ears of 'Autan' following low, early fertilization to $63{\cdot}2 \pm 2{\cdot}5$ in control ears of 'Apache' following high, early fertilization. The experimental conditions resulted in very large variations in both N_{ant} and G_n, but source and sink always remained correlated, except in the case of the trimming treatment. The overall correlation of N_{ant} to G_n was moderate, with $r^2 = 0{\cdot}46$ for $n = 72$ (Fig.1A), whereas estimates of culm N and grain number before trimming ($N_{ant,i}$ and $G_{n,i}$, respectively) were very strongly correlated (Fig. 1B), with the slope of the regression being significantly affected by genotype ($P < 0{\cdot}01$):

$$G_{n,i} = (1{\cdot}01 \pm 0{\cdot}06) \times N_{ant,i} + (18 \pm 2); \quad r^2 = 0{\cdot}90 \quad \text{for } n = 36 \text{ in 'Apache'} \qquad (3a)$$

$$G_{n,i} = (0{\cdot}75 \pm 0{\cdot}06) \times N_{ant,i} + (17 \pm 2); \quad r^2 = 0{\cdot}82 \quad \text{for } n = 36 \text{ in 'Autan'} \qquad (3b)$$

The amount of N per grain was significantly lower in 'Apache' than in 'Autan' ($P < 0{\cdot}001$; Table 3), but a highly significant interaction in genotype × ear reduction (G × R; $P < 0{\cdot}001$) was observed because this difference was no longer significant when trimming had been performed. In trimmed ears, the amount of N per grain increased up to $1{\cdot}7 \pm 0{\cdot}1$ mg grain^{-1} in both genotypes. The amount of N per grain was increased by late fertilization ($P < 0{\cdot}0001$), but not by early fertilization ($P > 0{\cdot}05$) A significant interaction in early fertilization × ear reduction (E × R; $P < 0{\cdot}01$), however, suggested that both increasing and high, early fertilization resulted in higher N amounts per grain, but only in trimmed ears.

N uptake after anthesis (N_{up}) ranged from 12 ± 2 to as much as 45 ± 3 mg culm^{-1} under the A and W treatments (see Table 2), respectively (Table 3), while its ratio to final N

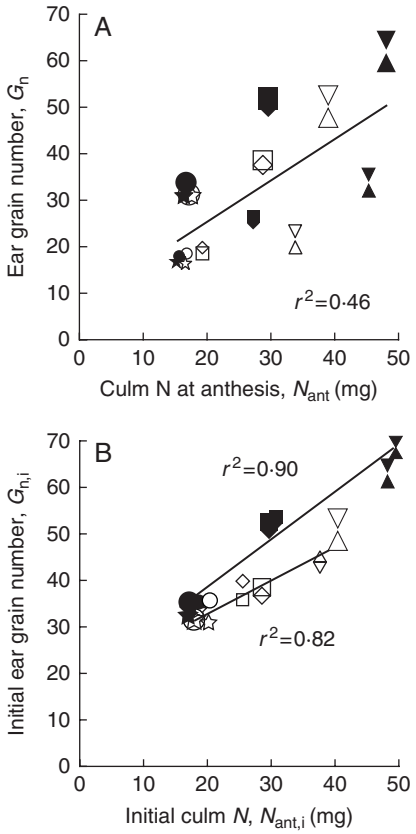

FIG. 1. Relationships between culm N at anthesis (including roots) and grain number. (A) Results after selected plants were trimmed at anthesis by removing one longitudinal half of the ear. (B) Genotype-dependant correlations between initial parameters before trimming. Each point is the mean of three pots containing two main culms. Closed symbols represent the genotype 'Apache' and open symbols the genotype 'Autan'; large symbols represent culms with whole ears and small symbols culms with trimmed ears. The various symbols refer to level of N fertilization before/after anthesis: low/low, circles; low/high, stars; medium/low, squares; medium/high, diamonds; high/low, triangles; high/high, inverted triangles. In (A) the regression line and correlation coefficient apply to the whole data set (d.f. = 71); in (B) two correlations are presented for the genotypes 'Apache' (closed symbols) and 'Autan' (open symbols).

yield ranged from 0·4 ± 0·1 to 0·9 ± 0·3. No significant effect of early fertilization on N_{up} was observed ($P > 0·05$), while late fertilization clearly enhanced N_{up} ($P < 0·01$). N uptake declined following ear trimming in 'Autan' (33 ± 3 vs. 22 ± 3 mg culm^{-1}), whereas it was not affected in 'Apache' (27 ± 2 vs. 26 ± 2 mg culm^{-1}). N uptake did not correlate with either N_{ant} or G_n ($r^2 < 0·12$ for $n = 72$).

The amount of N remobilized from vegetative parts after anthesis (N_{rem}) ranged from 7 to 36 mg culm^{-1}, and exhibited highly significant effects of genotype, early fertilization and ear trimming ($P < 0·0001$); only the effect of late fertilization on N_{rem} was not significant ($P > 0·05$). The average decrease in N_{rem} due to trimming was 3 ± 1 and 7 ± 2 mg culm^{-1} in 'Apache' and 'Autan', respectively. In 'Autan' therefore, but not in 'Apache', the decrease in N_{rem} due to trimming exceeded the amount of N in discarded spikelets. A highly significant G × E interaction ($P < 0·001$) was observed because the response of N_{rem} to early fertilization was stronger in 'Apache' than in 'Autan' (after low, increasing and high fertilization before anthesis, respectively: 10 ± 1, 19 ± 1 and 34 ± 2 mg culm^{-1} in 'Apache' vs. 9 ± 1; 15 ± 3 and 22 ± 2 mg culm^{-1} in 'Autan').

Lastly, N remobilization efficiency (NRE: ratio of N_{rem} to N_{ant}; Table 3) ranged from 0·28 ± 0·13 to 0·76 ± 0·02, and varied highly significantly with genotype and ear trimming ($P < 0·0001$). Indeed, highly significant G × R interactions ($P < 0·001$) were observed because trimming clearly reduced NRE in 'Autan', but hardly at all in 'Apache'. High, early fertilization increased NRE when compared to low, early fertilization ($P < 0·01$), whereas high, late fertilization decreased NRE ($P < 0·01$).

Determining N remobilization from vegetative parts

Figure 2A indicates that N_{rem} was very strongly correlated to N_{ant} ($r^2 = 0·91$; $n = 72$), with an intercept significantly different from zero. Analysis of variance of the residuals of this regression indicated that it was biased by genotype ($P < 0·0001$), early ($P < 0·01$) and late ($P < 0·001$) fertilization,

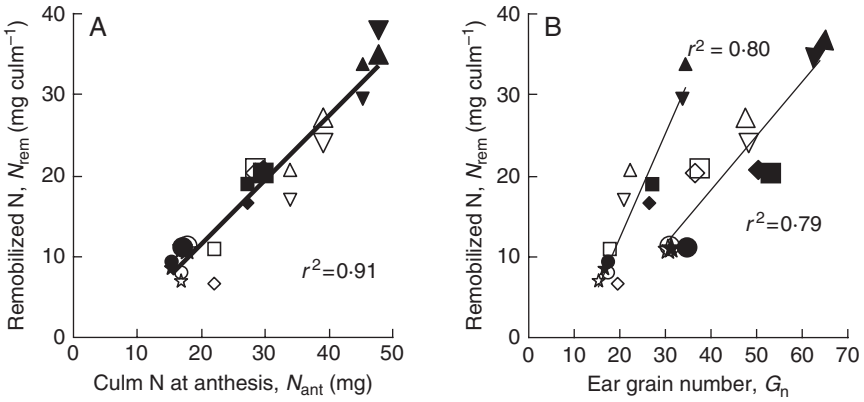

FIG. 2. Relationship between the amount of remobilized nitrogen from vegetative parts, including roots (N_{rem}) and (A) nitrogen in vegetative parts at anthesis, after trimming (N_{ant}), and (B) actual grain number (G_n). Each point is the mean of three pots containing two main culms. Symbols represent the various genotypes and treatments as in Fig.1. In (A) the regression line and correlation coefficient apply to the whole data set (d.f. = 71), while in (B) two correlations are given for whole ears (large symbols) and trimmed ears (small symbols).

as well as by ear trimming ($P < 0{\cdot}0001$). Quite clearly, N_{ant} is a major determinant of N_{rem}, but it is not alone. Grain number was also well correlated with N_{rem} (Fig. 2B), but a comparison of the regression lines indicates they were markedly affected by trimming treatment ($P < 0{\cdot}001$); the two regression lines are shown in Fig. 2, indicating r^2 at 0·79 and 0·80 ($n = 36$) for control and trimmed culms, respectively. However, analysis of variance of the residuals indicated that this trimming-dependent correlation was in turn biased by genotype ($P < 0{\cdot}01$) and early fertilization ($P < 0{\cdot}001$), but not by late fertilization ($P > 0{\cdot}05$). Lastly, despite very marked variations in N_{up}, within the same range as for N_{ant}, no correlation was observed when N_{up} was plotted against N_{rem} ($r^2 = 0{\cdot}03$, $n = 72$, data not shown).

Multiple regression analysis of N remobilization

N_{rem} was linearly correlated with the main determinants identified above, leading to the following multiple regression ($r^2 = 0{\cdot}94$ for $n = 72$):

$$N_{rem} = (0{\cdot}70 \pm 0{\cdot}04)N_{ant} + (0{\cdot}15 \pm 0{\cdot}03)G_n - (0{\cdot}07 \pm 0{\cdot}03)N_{up} - (4{\cdot}7 \pm 1{\cdot}0) \quad (4)$$

The coefficients for N_{ant} and G_n were positive and highly significant ($P < 0{\cdot}0001$), but the negative coefficient for N_{up} was much less significant ($P > 0{\cdot}01$). Lastly, the negative intercept of the relationship was clearly different from zero ($P < 0{\cdot}0001$). Such a correlation should be regarded with caution, however, as N_{ant} and G_n were far from being independent from each other. Moreover, the correlation exhibited bias for genotype and late fertilization ($P < 0{\cdot}01$), as well as a G × R interaction ($P < 0{\cdot}0001$): eqn (4) under-estimated N_{rem} in trimmed 'Apache' by $-1{\cdot}7 \pm 0{\cdot}2$ mg culm^{-1} and over-estimated N_{rem} in trimmed 'Autan' by $1{\cdot}8 \pm 0{\cdot}1$ mg culm^{-1}. In addition, the regression did not discriminate between genotypes despite the fact that their reaction to trimming was clearly different.

To obtain a sink variable that was less correlated to N_{ant} than G_n, the difference δG_n was then calculated between G_n and grain number, which could be predicted from $N_{ant,i}$ using eqn (3). Using δG_n instead of G_n, a new multiple regression analysis of N_{rem} was obtained (eqns 5a, b), which had a high level of significance ($r^2 = 0{\cdot}95$ for $n = 72$) and a constant that did not differ significantly from zero ($P > 0{\cdot}05$), provided the slope for δG_n was kept genotype-dependent:

$$N_{rem} = (0{\cdot}80 \pm 0.02)N_{ant} + (0{\cdot}05 \pm 0{\cdot}02)\delta G_n - (0{\cdot}11 \pm 0{\cdot}02)N_{up} \text{ in 'Apache'} \quad (5a)$$

$$N_{rem} = (0{\cdot}80 \pm 0{\cdot}02)N_{ant} + (0{\cdot}28 \pm 0{\cdot}03)\delta G_n - (0{\cdot}11 \pm 0{\cdot}02)N_{up} \text{ in 'Autan'} \quad (5b)$$

The coefficients for N_{ant} and N_{up}, as well as that for δG_n in 'Autan', were highly significant ($P < 0{\cdot}0001$), but this was not the case for δG_n in 'Apache' ($P > 0{\cdot}01$). The only significant bias ($P < 0{\cdot}01$) was observed for trimmed culms: in these plants, N_{rem} was under-estimated by $-1{\cdot}0 \pm 0{\cdot}5$ mg culm^{-1} under low, late fertilization and over-estimated by $1{\cdot}2 \pm 0{\cdot}5$ mg culm^{-1} under high, late fertilization. The genotype effect on the coefficient for δG_n suggested that there was almost no sink limitation in 'Apache', in contrast to 'Autan'; this is indicated in Fig. 3, which shows the respective contributions of the terms in eqn (5) to N_{rem}. As the root-mean-square error (RMSE) of the fit was low (2·1 mg culm^{-1}), the residuals of N_{rem} in its estimation by eqns (5a) and (5b) were within 8 % of the actual N_{rem} in all but one case (exhibiting a very low N_{rem}). The major role of N_{ant} in determining N_{rem} was clear, while N_{up} led to a decrease in N_{rem} from -1 to -5 mg culm^{-1}, generally accounting for between -10 % and -30 % of N_{rem}. Lastly, the influence of δG_n was markedly genotype-dependent in trimmed culms: the corresponding loss was -8 ± 1 % N_{rem} in 'Apache' vs. -60 ± 9 % N_{rem} in 'Autan'. In contrast, the influence of δG_n was very small in untrimmed plants ($\pm$ 0·5 mg culm^{-1}, less than 3 % N_{rem}), and in this case the multiple regression became genotype-independent, as the coefficient of δG_n did not significantly differ from zero for both genotypes ($P > 0{\cdot}05$), leading to the following, simpler equation ($r^2 = 0{\cdot}99$ for $n = 36$; RMSE = 1·6 mg culm^{-1}):

$$N_{rem} = (0{\cdot}77 \pm 0{\cdot}02)N_{ant} - (0{\cdot}08 \pm 0{\cdot}02)N_{up} \quad (6)$$

The coefficients for N_{ant} and N_{up} were then highly significant ($P < 0{\cdot}0001$ and $P < 0{\cdot}001$, respectively), without significant bias for either genotype or fertilization treatment, as suggested by analysis of variance for the correlation residuals (data not shown).

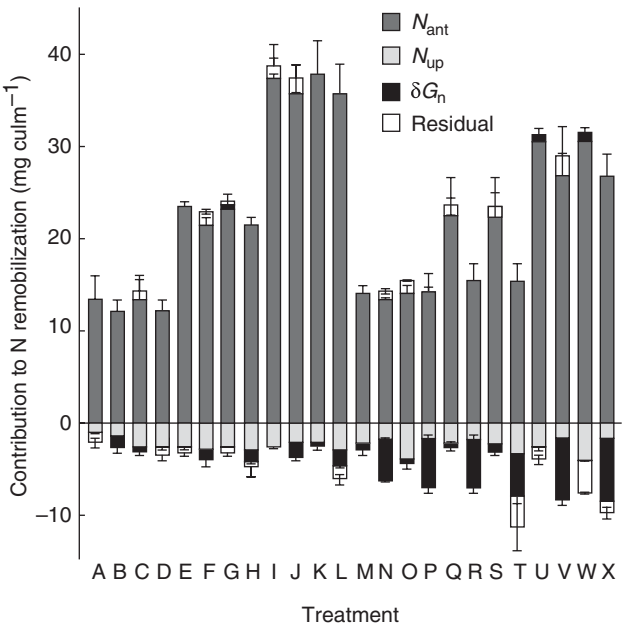

FIG. 3. Contribution to N remobilization in main culm (N_{rem}) for the various treatments as detailed in Table 2. The various terms used in eqn (5) are shown, as well as the residuals of the multiple correlation to N_{rem} of N_{ant}, N_{up} and δG_n. N_{ant} is the amount of N in vegetative parts at anthesis (after trimming), N_{up} is the N uptake after anthesis, and δG_n refers to the difference between G_n and grain number as predicted from $N_{ant,i}$ (before trimming) by eqn (3). Bars indicate the s.e.m. for three replicates.

TABLE 4. *(a) Importance of taking roots into account for the assessment of anthesis N, post-anthesis N uptake and N remobilization. Data are expressed as either mg per above-ground parts of the culm or relative to the results obtained using the whole culm. Data in this table can be compared with those in Table 3, which were obtained taking roots into account. (b) ANOVA was performed by fully crossing the effects of genotype, early fertilization, late fertilization and ear reduction, as well as first-order interactions between the different factors*

(a) N variables

	Above-ground anthesis N		Above-ground N increase		Above-ground N remobilization	
	Total (mg)	Ratio to whole culm	Total (mg)	Ratio to whole culm	Total (mg)	Ratio to whole culm
Genotype (G)						
'Apache'	26[a]	0·84[a]	30[a]	1·13[b]	18[b]	0·84[a]
'Autan'	23[a]	0·86[a]	30[a]	1·10[a]	13[a]	0·85[a]
Early fertilization (E)						
Low	14[a]	0·83[a]	25[a]	1·07[a]	8[a]	0·83[a]
Increasing	23[b]	0·85[a]	32[a]	1·08[a]	14[b]	0·86[a]
High	35[c]	0·85[a]	32[a]	1·18[b]	24[c]	0·84[a]
Late fertilization (L)						
Low	24[a]	0·85[a]	26[a]	1·13[a]	16[a]	0·85[a]
High	24[a]	0·85[a]	33[b]	1·01[a]	15[a]	0·85[a]
Ear reduction (R)						
Control	26[a]	0·85[a]	33[a]	1·10[a]	18[b]	0·86[a]
50 % trimming	23[a]	0·84[a]	26[a]	1·12[a]	13[a]	0·83[a]

(b) ANOVA

	Above-ground anthesis N		Above-ground N increase		Above-ground N remobilization	
	Total (mg)	Ratio to whole culm	Total (mg)	Ratio to whole culm	Total (mg)	Ratio to whole culm
G	–	–	ns	*	***	ns
E	***	ns	–	***	***	ns
L	nd	nd	*	–	ns	ns
R	–	ns	*	ns	***	–
G × E	–	ns	ns	ns	**	ns
G × L	nd	nd	ns	ns	ns	ns
G × R	ns	ns	–	ns	–	ns
E × L	nd	nd	ns	ns	ns	ns
E × R	ns	ns	ns	ns	ns	–
L × R	nd	nd	ns	ns	ns	ns

In (a) different letters indicate a significant difference between means ($P < 0{\cdot}01$; $n = 72$).

In (b) * $P < 0{\cdot}01$; ** $P < 0{\cdot}001$; *** $P < 0{\cdot}0001$; ns denotes non-significant effects ($P > 0{\cdot}05$); nd indicates that the effect of late fertilization did not apply to N_{ant}.

Predictions of remobilization without root measurements

All the data presented so far in this paper have involved the quantification of root N using the experimental design described. However, root sampling is impossible in field assays, and in such cases N_{ant}, N_{up} and N_{rem} are commonly estimated using $N_{ant,a}$, $N_{up,a}$ and $N_{rem,a}$, obtained by applying eqns (1) and (2) to above-ground parts rather than the whole culm. The values of $N_{ant,a}$, $N_{up,a}$ and $N_{rem,a}$ were affected by genotype, fertilization and ear trimming, in a way similar to N_{ant}, N_{up} and N_{rem}, respectively (Table 4). Both $N_{ant,a}/N_{ant}$ and $N_{rem,a}/N_{rem}$ ratios averaged 0·85, with variations that were not significantly linked to genotype, fertilization or ear trimming. NRE and its estimation using the $N_{rem,a}/N_{ant,a}$ ratio did not differ significantly according to a paired sample comparison ($P > 0{\cdot}05$). The value of $N_{up,a}$ (Table 4) considered remobilization from the roots as an N uptake, and therefore over-estimated N_{up} by between 1 % and 27 %, depending on genotype ($P < 0{\cdot}01$) and early fertilization ($P < 0{\cdot}0001$), but not on late fertilization ($P > 0{\cdot}01$) or ear trimming ($P > 0{\cdot}05$). Therefore, N_{up} could not be precisely evaluated from $N_{up,a}$ measurements in above-ground parts only. Nevertheless, a direct estimate of N_{rem} in untrimmed culms was obtained from $N_{ant,a}$ and $N_{up,a}$ ($r^2 = 0{\cdot}97$ for $n = 36$; RMSE = 1·8 mg culm^{-1}):

$$N_{rem} = (0{\cdot}91 \pm 0{\cdot}03)N_{ant,a} - (0{\cdot}07 \pm 0{\cdot}02)N_{up,a} \quad (7)$$

Despite it being less relevant from a physiological point of view, the following regression (eqn 8), also restricted to untrimmed culms, could be more easily tested in a field experiment and even compared with previous studies ($r^2 = 0{\cdot}97$ for $n = 36$; RMSE = 1·3 mg culm^{-1}):

$$N_{rem,a} = (0{\cdot}78 \pm 0{\cdot}02)N_{ant,a} - (0{\cdot}06 \pm 0{\cdot}02)N_{up,a} \quad (8)$$

In both eqns (7) and (8), the coefficients for $N_{ant,a}$ and $N_{up,a}$ were both highly significant ($P < 0{\cdot}0001$ and $P < 0{\cdot}001$, respectively), without any significant bias regarding genotype,

fertilization treatment or interactions, as suggested by analysis of variance of the regression residuals. It appeared that eqn (8) was indistinguishable from eqn (6), except that it referred to above-ground values.

DISCUSSION

Representativeness of NRE

This experiment was designed to provide for very large N_{up} by inducing N deficiencies before anthesis (Triboï and Triboï-Blondel, 2002), so that the ratio of N_{up} to grain N ranged from 0·4 to 0·9 while the values attained in crops are commonly below 0·5 (Van Sanford and MacKown, 1987). However, despite exploring unusual ranges for the N_{up}/N_{rem} ratio, the usual values for N_{ant} and N_{rem} were also represented in the study. Previous field experiments have reported higher values for NRE (0.4 to 0.9) than those found during the present study (0·3 to 0·8). Kichey *et al.* (2007) suggested a genotype effect on NRE, but Barbottin *et al.* (2005) noted that the genotype actually interacted with the year and level of fertilization. For this reason, the low NRE levels obtained in the present study could hardly be explained by the choice of cultivars. Unlike field experiments, this study also took roots into account, which are a major N sink according to Andersson *et al.* (2004). However, despite the fact that root N varied considerably from 7 % to 27 % of N in vegetative parts (depending on the genotype and treatment), the trends observed in whole culms were essentially maintained when only above-ground parts were examined. The sampling with roots therefore may not provide an explanation for the discrepancy between the results of this study and those in the literature.

The absence of high NRE values may have originated from the use of measurements on the scale of the culm, rather than the m^2 scale as reported in the literature. The relationships between N_{ant} and N_{rem} appeared to be affected by the scale considered. At the m^2 scale, the simple linear regression of N_{ant} to N_{rem} shows a positive intercept (e.g. in Barbottin *et al.*, 2005). Consequently a higher N_{ant} thus mathematically led to a higher NRE, which may have occurred when different levels of fertilization prior to anthesis were compared. The higher the fertilization, the higher was N_{ant} and the lower was the NRE, as previously noted by Cox *et al.* (1986). In contrast, the data in this paper suggest that the simple linear regression of N_{ant} to N_{rem} displayed a negative intercept at the culm scale. Therefore a higher N_{ant} thus mathematically led to a lower NRE, leading to an effect of early fertilization in contradiction to findings in the literature (Table 3). In fact, Fig. 2A indicates that N_{ant} and N_{rem} are aligned in the overall correlation; thus the variation in NRE was only based on the negative intercept of the relationship between N_{ant} and N_{rem}. It is possible to imagine that a certain level of N will be immobilized in the dead tissues of a single culm by the time that anthesis occurs. This would result in the negative intercept observed at the culm level. At a later stage, grain N filling would lead to N remobilization from senescing (but still alive) plant organs. The present data suggest that the corresponding slope, which could be termed the 'physiological NRE', was unaffected by fertilization. The ratio of N_{rem} to N_{ant}, which could be termed the 'culm NRE', was, however, biased by the intercept of the correlation, and increased with fertilization. At the m^2 level, the relationships between N_{ant} and N_{rem} become still more complicated because of tillering, resulting in a positive intercept and a 'crop NRE' that decreases with fertilization. Therefore, the use of the NRE could lead to confusion.

N_{rem} *was essentially source-determined*

The principal determinant for N_{rem} was by far N_{ant}, with slopes in different equations of around 0·75–0·80. This result agreed well with that of Barbottin *et al.* (2005), who reported that, over a very broad range of N_{ant}, the slope of N_{rem} vs. N_{ant} was 0·76 under simple regressions regardless of genotype, provided that neither important fertilization was applied at anthesis nor that stresses occurred thereafter. The data also indicated a weaker correlation between N_{rem} and G_n, which could be associated with the link between N_{ant} and G_n. Studies in the literature have reported a correlation between N_{ant} and G_n at the m^2 level, although this was mostly linked to the degree of tillering. This correlation was also observed at the main-culm level, where it was genotype-dependent: 'Autan' produced less grain than 'Apache', even for the same N_{ant}. This suggests that the plants themselves somehow regulate their sink capacity (G_n) to their source level (N_{ant}) around anthesis (Sinclair and Jamieson, 2006). The use of the δG_n difference rather than G_n to characterize the sink led to a reduction in the sensitivity of N_{rem} determinism to both sink and genotype; in untrimmed culms their effects became negligible. The absence of a constant in eqns (6)–(8), which could therefore probably be extended to the m^2 level, indicated that N_{rem} was fully predicted using source data only (N_{ant} and N_{up}). However, it did not mean that sink and genotype had no influence on grain N filling, but rather that the sink influence was taken into account by the genotype-dependent relationship through G_n and N_{ant}. Any event disturbing this relationship, either before or after anthesis, could lead to reintroduction of both the genotype and sink effects, for instance when disease or water stress occurr during grain filling, as was observed by Barbottin *et al.* (2005). Sink and genotype effects also appeared in the response to ear halving, but multiple regressions suggested that the source remained the principal determinant of N_{rem}, although modulated by sink and genotype. On the other hand, in many cases explicitly accounting for the sink may be redundant, as Jamieson and Semenov (2000) showed that it is simpler to fit grain N filling to sources than to sinks. If regulation somehow occurs, it should be located in sources themselves; according to Hörtensteiner and Feller (2002) and Gregersen and Holm (2007) vegetative parts seem to regulate N_{rem} themselves by means of a generic senescence program. Nevertheless, despite numerous models in the literature that have described grain N filling regulation by source/sink interactions, to my knowledge no groups have yet described how this regulation could be attained otherwise.

Sink effects in trimmed ears

Ear trimming always resulted in an increase in the amount of N per grain, even though it reduced N yield per ear in

most cases. As early as 1991, Jenner *et al.* suggested that the relationship between source availability and sink activity shifts gradually as a function of the range of the source/sink ratio. If this ratio is low, grain filling would be proportional to source activity, approaching a constant-rate, saturated pattern with higher source availability. These authors also suggested that the dry-matter filling of grain is similar to a saturated pattern, while the grain N filling of whole ears tends to be within the range of a proportional pattern. Ear trimming would thus shift grain N filling to the range of the source/sink ratio leading to a saturated pattern. Bancal and Soltani (2002) modelled this type of mixed source and sink determinism, but only in the case of partitioning between several sinks. In fact, grain N filling is too frequently considered to be a single-sink task, whereas ^{15}N labelling studies have indicated that N absorbed after anthesis may actually be incorporated, and not just temporarily stored, in vegetative parts (Oscarson, 1996; Kichey *et al.*, 2007), thus suggesting that vegetative parts also behave as a sink for newly absorbed N. The model described by Bancal and Soltani (2002) used at least two parameters per sink that mostly affected sink activity at either low or high source/sink ratios, respectively. These parameters might vary according to genotype, which could also explain the genotype effect on trimming response suggested by the genotype-dependent coefficient for δG_n. According to Martre *et al.* (2003), genotypes exhibiting a larger amount of N per grain in untrimmed ears reached their saturation level earlier following trimming, which was also observed during this study in 'Autan' vs. 'Apache'. These hypotheses would not preclude alternative models explaining a trimming effect due to carbon (Dingkuhn *et al.*, 2007) or hormones (Yang, *et al.*, 2003). These puzzling results obtained for trimmed ears should be extended to control plants only with caution.

Negative effect of N_{up} *on* N_{rem}

The negative effect of late fertilization on NRE has long been recognised (Gooding *et al.*, 2007); however, labelling studies have suggested that remobilization is not actually inhibited but counterbalanced by the incorporation of newly absorbed N in vegetative tissues. The harvest index of N absorbed after anthesis is lower than 100 %. Using ^{15}N fertilizers, Kichey *et al.* (2007) reported that 89·7 % to 93·4 % of this N was translocated to grains, while under eqn (6) of this study the difference from 1 for the slope of N_{rem} to N_{up} suggested that 92 ± 2 % of N absorbed after anthesis was translocated to grains in untrimmed ears. Therefore, the unusually high ratios of N_{up} to grain N did not diminish translocation efficiency. Current trends towards late fertilization or plant breeding to ensure continued N uptake would therefore not result in high levels of N waste in straw.

Conclusions

In untrimmed ears, N yield could be predicted accurately without data for grain number. Sink and genotype effects were only modulations of the major regulation by both sources N_{ant} and N_{up}, and moreover they only appeared in response to severe stresses (such as ear halving). The results obtained on trimmed ears thus differed qualitatively from the others and should therefore be extended to control plants with caution. N_{rem} was positively correlated with N_{ant} and negatively with N_{up}, but even with a very high N_{up} the negative impact of N_{up} on N_{rem} remained small. It will therefore continue to be useful to investigate how the uptake of late nitrogen fertilization can be increased.

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