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Effect of nitrogen fertilizer application timing on nitrogen use efficiency and grain yield of winter wheat in Ireland

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Abstract

The objectives of this work were to determine the effects of initiating application of fertilizer nitrogen (N) to winter wheat at different growth stages (GSs) on grain yield and N use efficiency (NUE). A factorial experiment was carried out in two growing seasons (2011 and 2012) with five timings of first N application (GS 24/26 [tillering], GS 30, GS 31, GS 32 or GS 37) and an unfertilized control, two sowing densities (100 and 400 seeds/m²) and a cattle slurry treatment (with or without slurry). The latter was included to simulate variation in soil N supply (SNS). Delaying the first application of N from the tillering stage until GS 30 had no significant effect on grain yield in either year. Further delaying the initial N application until GS 31 caused a significant yield reduction in 2011, in comparison to GS 30 application, but not in 2012. Differences in efficiency of recovery and use of fertilizer N by the crop among the first three application timings were small. There was no evidence to support alteration in the timing of the first application of N in response to low plant density. Slurry application did not influence SNS, so the interaction between SNS and fertilizer N application timing could not be determined. It is concluded that in order to maximise yield and NUE, the first N application should be applied to winter wheat between late tillering and GS 30 and that delaying the first N until GS 31 can lead to yield reductions compared to the yield obtained with earlier application.

Keywords

application timing • fertilizer efficiency • grain yield • nitrogen fertilizer • wheat

Introduction

Nitrogen (N) is an essential macronutrient for wheat production. Soils can supply a considerable amount of N to a wheat crop as a result of mineralisation of N from the soil organic matter, a process that takes place both prior to and during the crop growth period (Sylvester-Bradley *et al.*, 2001). However, under the high yielding conditions experienced in Ireland, application of fertilizer N is normally necessary to supplement available soil N to fulfil the crop's requirement. For both economic and environmental reasons, efficient use of N is critical and matching N supply with crop demand, in both space and time, is an effective means of achieving efficient use of N (Zebarth and Rosen, 2007; Cui *et al.*, 2010). Soil N is normally sufficient to supply a crop's needs over the winter period and recovery of fertilizer N applied in the autumn/winter period is generally low; consequently, yield response to these applications is often low (Ellen and Spiertz, 1980; Powlson *et al.*, 1986). As temperatures increase in spring, crop growth, and hence the crop N demand, increases such that soil N is no longer sufficient to supply the crop's needs. If the soil inorganic N pool is not supplemented with fertilizer N, crop growth and yield will be reduced. The stage

at which soil N becomes limiting, and therefore when fertilizer N is required, depends on the amount of N in the soil and, therefore, the optimum timing of the initial application of N is likely to be influenced by the levels of available soil N.

In addition to being available for crop uptake, inorganic N in the soil, originating either from fertilizers or from mineralisation of soil N, is subject to the process of immobilisation. Normally, levels of inorganic N decline relatively quickly (within 2–3 wk) after fertilizer N application as a result of either crop uptake or immobilisation (Recous and Machet, 1999). Therefore, applying fertilizer N in excess of the crop's immediate requirement can result in a proportion of that N becoming unavailable to the crop through immobilisation, leading to reduced recovery of fertilizer N by a crop (King *et al.*, 2001). This indicates that making the first application of N to a winter wheat crop too early, while the crop can still acquire adequate N from soil reserves, could lead to inefficient recovery of that fertilizer N by the crop.

A number of studies have found reduced recovery of N applied at tillering compared to that applied later in the growth cycle (Recous *et al.*, 1988; Limaux *et al.*, 1999; Blankenau *et al.*,

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2002; Howard *et al.*, 2002). Therefore, applying fertilizer N when crop growth rates are such that there is a high demand for N increases the recovery of fertilizer N in the crop and also the grain yield (Fischbeck *et al.*, 1990; Limaux *et al.*, 1999). Consequently, N from fertilizer applications that are applied later in the growth cycle will be more efficiently recovered by a crop than earlier applications (Recous and Machet, 1999). Petersen (2004) concluded that the increased recovery of fertilizer N with delayed application was due to increased crop demand, which was linked to greater rates of leaf area production.

However, high rates of fertilizer N recovery are only advantageous if that N is efficiently converted into yield. Yield is closely related to intercepted solar radiation, which in turn is related to the amount of photosynthetic area of the crop (Gallagher and Biscoe, 1978). The photosynthetic area in turn is related to the N accumulation of the crop, so that N deficiency during the period of green area production can lead to reductions in yield (Sylvester-Bradley *et al.*, 1990; Grindlay *et al.*, 1997). Therefore, although delaying N fertilizer application may improve fertilizer N recovery in the crop, there may be adverse effects on aspects of the crop necessary for production of yield, such as the production of leaf area and number of grains per unit area during the early growth stages (GSs). Deciding on the date to initiate fertilizer N application can involve a trade-off between N recovery and efficiency of use of accumulated N in terms of yield production.

In Ireland, the current recommended timing for the first application of fertilizer N to winter wheat is between late tillering and the onset of stem elongation (Coulter and Lalor, 2008). In practice, many growers apply N in late February/early March, often in advance of significant spring growth, which may be leading to reduced nitrogen use efficiency (NUE). Fischbeck *et al.* (1990) indicated that excess N applications before the wheat crop began stem extension, particularly in areas where there was high N supply from the soil, could lead to reductions in yield. Relatively little work has been carried out regarding the optimum timing of the initial application of fertilizer N to wheat under Irish conditions. Fewer (2002) reported that there was no significant effect on grain yield when delaying the initial application to winter wheat until GS 31, where the wheat was grown after a grass ley and where N supply from the soil in spring is likely to have been high. However, a significant reduction in yield was observed when the initial N was delayed until GS 31 when the wheat crop was grown after three years of arable cropping, where N supply from the soil was lower than that from the site following a grass ley. This suggests that the level of soil N supply in early spring may be important when deciding the stage at which to initiate fertilizer N application. In Belgian conditions, Bodson *et al.* (2001) concluded that in many situations, delaying the initial N application until GS30 had no significant effect on grain yield, but in certain situations, particularly in which root growth was

restricted, delaying the initial N could cause yield reduction. The objectives of this work were: (a) to determine the effects of initiating fertilizer N application to winter wheat at different GSs on grain yield and NUE of the fertilizer N and (b) to determine whether plant population density or soil N supply potential influenced the stage at which the first N application should be applied.

Materials and methods

Experimental sites

Two field experiments were carried out at the Teagasc Crops Research Centre, Oak Park, Carlow, Ireland (52.86° N, 6.92° W, 54 m above mean sea level): one in the 2010/11 growing season and one in the 2011/12 growing season (henceforth referred to as 2011 and 2012, respectively). The soil at both sites is a grey-brown Podzolic of the Mortarstown Series (Conry and Ryan, 1967). Both sites had been cultivated for at least 20 yr prior to the initiation of the experiments; the previous crops were winter oats and winter oilseed rape for the 2011 and 2012 experiments, respectively.

Experimental layout, design and treatments

A split-plot experimental arrangement, laid out in a randomised complete block design with four replications, was used for both experiments. To facilitate repeated destructive sampling, each split plot comprised two adjacent plots (12 m × 2.15 m), one of which was designated for destructive sampling and the other reserved for yield determination. In both seasons, each plot had 14 rows spaced 15.4 cm apart.

The main plot factor was slurry treatment; there were two levels (with and without slurry). The slurry treatment was included to simulate a higher level of soil N supply (SNS) compared to the treatment in which slurry was not applied. Cattle slurry was applied, using a tractor-drawn trailing hose tanker system, to the stubbles of the previous crop and immediately (within 1 h) incorporated by ploughing. Total N applied in the slurry (22 m³/ha containing 2.35 kg N/m³) was 51.7 kg/ha in 2011 and 2012. Sowing of the wheat crop took place within 2 d of slurry application in both seasons. To ensure that any effect of the slurry would be mainly due to its effect on SNS to the crop, P and K fertilizer was applied to the no-slurry plots to balance the estimated P and K inputs in the slurry to the slurry-treated plots.

The split-plot treatments included a factorial arrangement of N regime (five fertilizer N application timing treatments and an unfertilized control [0N]) and seed rate (high and low). Seed rate was included as a factor to give variation in plant population density. The seed rates used were 400 seeds/m² and 100 seeds/m², to give plant population densities sufficient to saturate the yield response to increasing seed

rate and a suboptimal plant population density, respectively (Spink *et al.*, 2000).

The N application timing treatments were defined by the GS (Zadoks *et al.*, 1974) at which the first of a total of two applications of fertilizer N was applied. Five fertilizer N application timing treatments, where the first N fertilizer was applied at GSs 24–26, 30, 31, 32 or 37 (hereafter referred to as Ntillering, N30, N31, N32 and N37) were included (Table 1). A control treatment receiving no fertilizer N (0N) was also included. Each treatment received 70 kg N/ha in the first fertilizer application. A second application of 130 kg N/ha was made 15–27 d after the first application but not before GS 31 in the case of Ntillering and N30 to prevent excessive N applications before significant crop growth occurred in these treatments. Calcium ammonium nitrate was used as the N source. Fertilizer was applied using a carefully calibrated modified seed drill (Fiona Maskinfabrik A/S, Bogense, Syddanmark, Denmark).

Winter wheat (cv. Cordiale) was sown on 12 October 2010 and 14 October 2011. Herbicides, fungicides and insecticides were applied uniformly across the trials according to standard farm practice to maintain the crop free of weeds, fungal disease and insect pests. Nutrients, other than nitrogen, were applied uniformly to the experiments according to the requirements of the crop (Coulter and Lalor, 2008).

Sample collection and analysis

Samples were taken on three occasions in each season: at GS 30 (26 March 2011, 30 March 2012), at anthesis (GS 61-65; 12 June 2011, 18 June 2012) and at maturity (9 August 2011; 13 August 2012), just prior to combine harvest. At GS 30, samples were only collected from the unfertilized treatment. All plots were sampled at anthesis and maturity. On

each occasion, plants from two adjacent 0.5 m row lengths at two random locations (equivalent to a sampling area of 0.308 m²) were sampled. Roots were removed and samples were dried in a force-ventilated oven for 3 d at 70°C and dry matter (DM) yield determined.

At maturity, prior to oven-drying, the total number of ears in each sample was determined and expressed as ears/m². Samples were then threshed, using a laboratory threshing machine, and the straw, grain and chaff were collected separately. Thousand grain weight (TGW) was determined for each sample using an electronic grain counter (Contador; Pfeuffer, Kitzingen, Germany) and expressed as 85% DM after oven-drying. The mean number of grains per ear (GPE) was calculated by dividing the total number of grains in each sample by the number of ears in each sample.

Samples were subsequently milled (< 2 mm particle size) using a laboratory grinder (SM100; Retsch, Haan, Germany) and total N concentration determined using a Leco FP 328 instrument (LECO Corporation, St. Joseph, MI, USA) calibrated using an ethylenediaminetetraacetic acid standard. Total N accumulation for each sampling date was calculated as the product of DM yield and the N concentration (grain N concentration was calculated from grain protein concentration using a conversion factor of 5.7). At harvest, the N accumulation levels for straw, chaff and grain were summed up to give the total N accumulation.

At maturity, grain yield was determined using a Deutz-Fahr combine harvester modified for plot harvesting with on-board electronic weighing equipment. A subsample of grain (~ 2 kg) was taken from each plot, and moisture and crude protein contents were determined using a near infra-red spectroscopy instrument (Infratec 1241; Foss A/S, Hillerød, Denmark). Grain yield (t/ha) was expressed at 15% moisture.

Table 1. Dates and growth stages at which fertilizer N was applied

N application timing	First application		Second application	
	Date	GS ¹	Date	GS ¹
2011				
Ntillering	Mar 9	GS 26	Apr 11	GS 31
N30	Mar 28	GS 30	Apr 15	GS 31
N31	Apr 11	GS 31	Apr 27	GS 33
N32	Apr 20	GS 32	May 10	GS 39
N37	May 10	GS 39	May 24	GS 55
2012				
Ntillering	Feb 29	GS 24	Apr 4	GS 31
N30	Mar 23	GS 30	Apr 19	GS 32
N31	Apr 4	GS 31	Apr 19	GS 32
N32	Apr 19	GS 32	May 4	GS 32
N37	May 14	GS 37	May 25	GS 51

¹GS, growth stage.

Calculations

Efficiency ratios of fertilizer N use for the harvest sampling date were calculated according to Ladha *et al.* (2005).

$$\text{Recovery efficiency of N (RE}_n\text{)} = (U_f - U_0)/F_n$$

$$\text{Agronomic efficiency of N (AE}_n\text{)} = (Y_f - Y_0)/F_n$$

$$\text{Physiological efficiency of N (PE}_n\text{)} = (Y_f - Y_0)/(U_f - U_0)$$

where U_f and U_0 are the total N accumulation levels (kilograms N per hectare) and Y_f and Y_0 are the grain yields (kilograms dry matter per hectare) in the fertilized and unfertilized treatments, respectively, and F_n is the fertilizer N application rate (kilograms N per hectare).

Statistical analysis

A combined analysis of variance over the two years was carried out for each variable. Data were analysed using the MIXED procedure of SAS 9.1 (SAS, 2004). Year, seed rate, slurry application, N regime and their interactions were included as fixed effects; replication and replication × slurry were included as random effects. When significant ($P < 0.05$) effects of the fixed effects or their interactions were found, least-square means were separated using the Tukey–Kramer multiple comparison test.

Results

Grain yield and yield components

Grain yield in the 0N samples did not differ significantly between the two years (Figure 1). For the fertilized treatments, yields in the Ntillering, N30 and N31 samples were significantly higher in 2011 than in 2012, while the yields in N32 and N37 did not differ significantly between years.

All fertilized treatments had significantly higher yield than the 0N sample in both years (Figure 1). N application timing had a significant effect on grain yield, although the effects of N application timing varied between years, as indicated by a significant interaction between N regime and year (Table 2). In 2011, delaying the first N application until GS 30 or GS 31 gave similar yields, compared to the yields after first N application at tillering. Delaying the first application of fertilizer N until GS 31 caused a significant reduction in yield (0.7 t/ha) compared to that obtained after first N application at GS 30. However, yield at N31 was not significantly different from that at Ntillering. Each delay in the first N application after GS 31 caused a significant reduction in yield of at least 0.9 t/ha. In 2012, similar yields were achieved irrespective of whether the first N application was at tillering, GS30, GS 31 or GS32. A significant reduction (0.9 t/ha) was observed when the first N application was delayed from GS32 to GS37 (Figure 1). N37 gave the lowest yield in both years. It was noted that tillers that had not reached maturity were prevalent in the N37 treatment, but not in any other treatment, at harvest.

Correlation analysis indicated that yield differences between N application timing treatments in 2011 (i.e., excluding 0N) were due to the effects of the treatments on both GPE and TGW (Table 3). Ear density was not correlated ($r = -0.20$, $P = 0.07$) with yield in 2011. In 2012, differences in yield were due to differences in TGW and ear density, but the lower correlation coefficients indicated that the relationships were weaker than in 2011.

There was a significant effect of seed rate on yield; increasing the seed rate from 100 seeds/m² to 400 seeds/m² gave a significant increase in mean yield of 0.36 t/ha (data not presented). No significant interaction between N regime

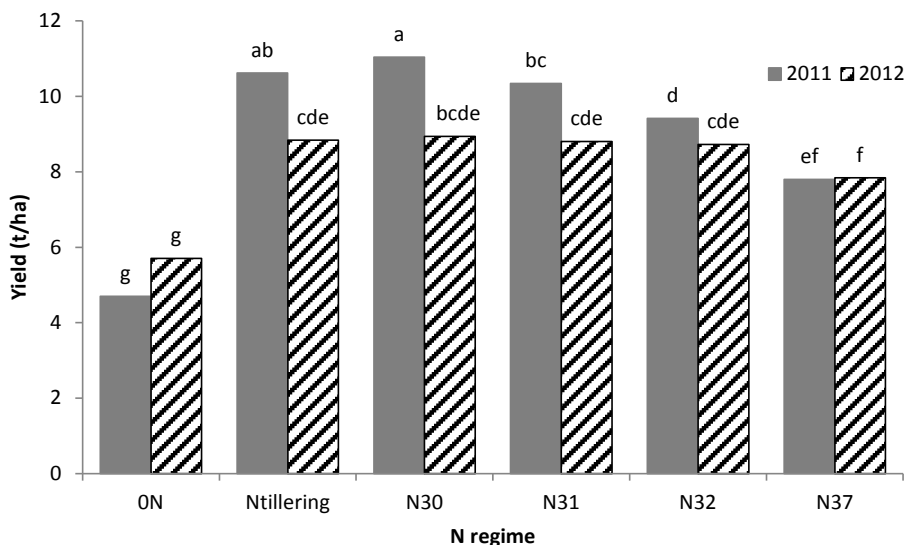


Figure 1. Effect of N regime on yield (t/ha at 85% DM) in 2011 and 2012. Bars with the same letter are not significantly different ($P > 0.05$).

Table 2. Tests of fixed effects for yield, components of yield, N accumulation and N efficiency ratios

Effect	Yield	Components of yield			N accumulation			N efficiency ratio			Grain N concentration
		GPE ¹	TGW (g) ²	Ears/m ²	March	Anthesis	Harvest	RE _{N3}	AE _{N4}	PE _{N5}	
N regime (N)	***	***	***	***	-	***	***	**	***	***	***
Slurry (S)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Seed rate (R)	***	***	NS	***	**	**	**	NS	**	*	***
Year (Y)	NS	*	***	**	***	**	NS	NS	**	***	***
N × Y	***	***	***	***	-	**	NS	NS	***	***	***
S × Y	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	**
R × Y	NS	*	NS	**	NS	NS	NS	NS	*	*	**
N × S	NS	*	**	NS	-	NS	NS	NS	NS	NS	NS
N × R	NS	**	NS	NS	-	NS	NS	NS	NS	NS	NS
S × R	NS	NS	NS	*	NS	NS	NS	*	**	NS	NS
N × S × R	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	NS
N × S × Y	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	NS
N × R × Y	NS	NS	NS	***	-	NS	NS	NS	NS	NS	NS
S × R × Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
N × S × R × Y	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	NS

¹GPE, grains per ear; ²TGW, thousand grain weight; ³RE_N, recovery efficiency of N; ⁴AE_N, agronomic efficiency of N; ⁵PE_N, physiological efficiency of N.

NS indicates non-significant results.

Table 3. Effect of N regime on components of winter wheat grain yield in 2011 and 2012

N regime	TGW (g)		GPE		Ears/m ²	
	2011	2012	2011	2012	2011	2012
0N	50.4 bc	40.3 e	31.2 b	44.8 a	282.1 e	349.8 d
Ntillering	55.3 ab	40.5 e	48.0 a	45.6 a	444.2 bc	532.7 a
N30	55.0 a	40.2 e	50.3 a	47.4 a	429.4 c	519.3 a
N31	53.2 ab	40.7 de	50.0 a	48.6 a	405.0 cd	495.9 ab
N32	50.0 bc	39.8 e	45.6 a	49.4 a	435.1 bc	530.0 a
N37	46.4 dc	39.2 e	28.8 b	49.1 a	539.6 a	458.8 bc
<i>r</i> ¹	0.77	0.32	0.50	-0.06	-0.20	0.35
<i>P</i> ²	***	**	***	NS	NS	**

¹*r* is the Pearson correlation coefficient between the component of yield for the respective year and grain yield for the N application timing treatments (excluding 0N).

²*P* indicates the significance of the correlation.

For each component of yield, means followed by the same letter are not significantly different.

and seed rate was detected, indicating that seed rate did not influence N regime effects (Table 2).

Nitrogen accumulation

Slurry application had no significant effect on N accumulation in March, at anthesis or at harvest (Table 2). The higher seed rate gave significantly higher N accumulation at all three sampling dates, but the differences (9.3 kg N/ha, 9.2 kg N/ha and 8.5 kg N/ha in March, at anthesis and at harvest, respectively) were small. N accumulation was significantly

greater both in March (15.6 kg N/ha in 2011 and 29.3 kg N/ha in 2012) and at anthesis in 2012 compared to the same periods in 2011, but there was no significant difference between the years at harvest.

Fertilizer addition, irrespective of timing of the first application, significantly increased N accumulation in both years at both anthesis and harvest (Figure 2). Differences were detected between the results at anthesis and harvest after different N application timings. At anthesis, the effect of N application timing was influenced by year. This reflected a greater

reduction in N accumulation as the initial fertilizer N application was delayed in 2011 than in 2012 such that the difference in N accumulation between Ntillering and N37 was much greater in 2011 than in 2012.

At harvest, there was no significant interaction between N regime and any other effect in terms of N accumulation (Table 2). There was a trend towards increased N accumulation as first N application was delayed. However, there was no statistically significant difference in N accumulation at harvest between the first four N application timings; N accumulation for N37 was significantly higher than that for Ntillering (Figure 2).

Seed rate or slurry application did not influence the effect of N application timing on N accumulation at harvest.

N use efficiency

Effects of N application timing on N accumulation were mirrored in the RE_N, with a trend towards higher RE_N as the first N application was delayed, but a significant difference was only detected between N37 and Ntillering (Table 4). Effects of N application timing on RE_N were not significantly influenced by either seed rate or slurry application. RE_N did not differ significantly between years.

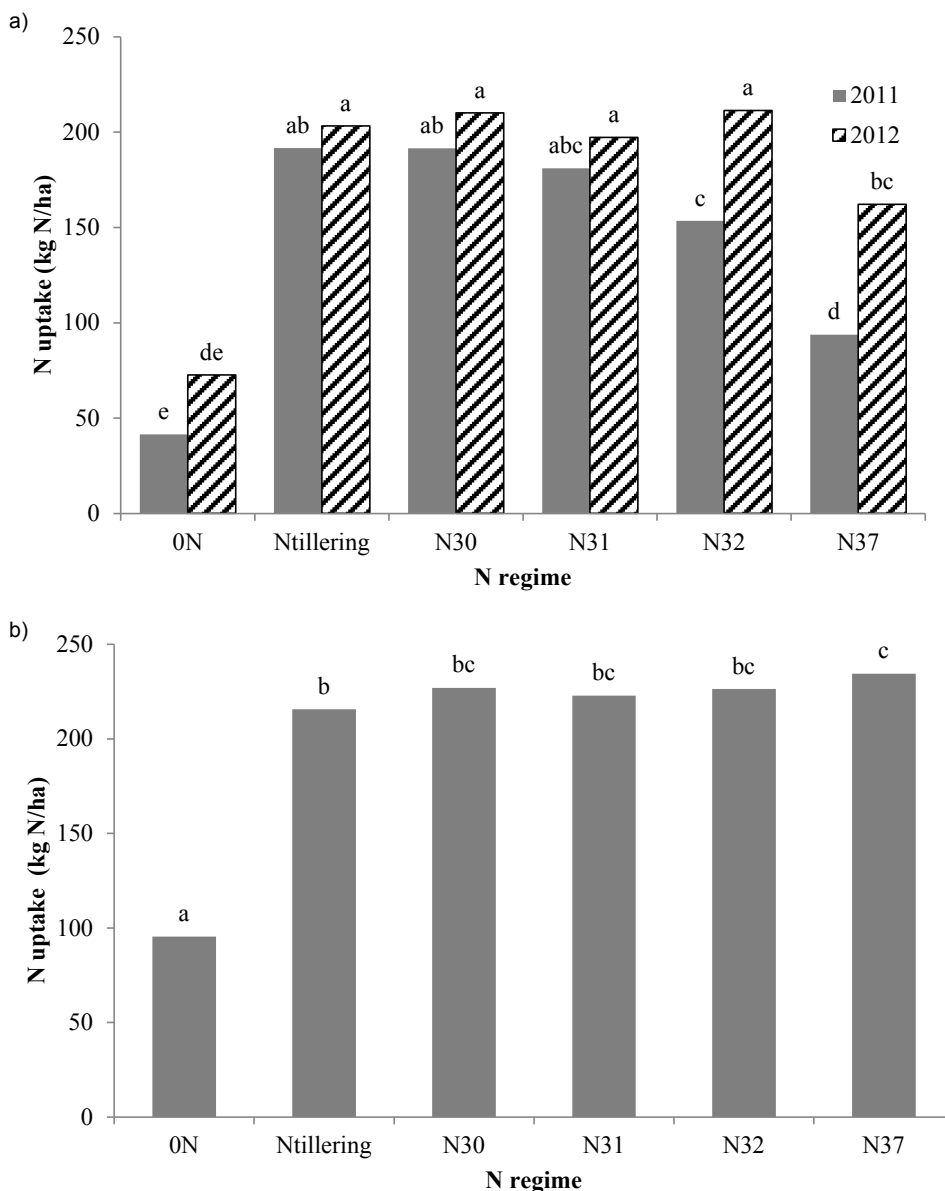


Figure 2. Effect of N regime on N accumulation of winter wheat (cv. Cordiale) at a) anthesis and b) harvest. Bars with the same letter are not significantly different.

Slurry addition had no significant effect on RE_N (Table 2). RE_N was significantly lower at the lower seed rate, but not at the high seed rate, where slurry was applied.

Agronomic efficiency (AE_N), the increase in yield relative to that of the unfertilized control per kilogram fertilizer N applied, was significantly affected by both N regime and year, but a significant interaction between N regime and year indicated that the effect of N regime varied between the two years (Table 2). AE_N was significantly higher for all N application timings, except N37, in 2011 compared to 2012, reflecting the higher yields achieved in 2011 (Table 4). In 2011, differences in AE_N were small among the first three N application timings, but declined significantly with each successive delay in initial fertilizer N application after GS 31. In 2012, there was no significant difference in AE_N between the first four application times, but a significant decline occurred when the first N application was delayed from GS 32 to GS 37.

The effect of N application timing on AE_N was not influenced by slurry application or seed rate (Table 2). Increasing seed rate significantly increased AE_N in 2012 but not in 2011. The effect of seed rate was also influenced by slurry application; slurry application reduced AE_N at the low seed rate but not at the high seed rate.

Slurry application had no significant effect on PE_N (Table 2). The higher seed rate gave significantly higher PE_N than the low seed rate in 2012 but there was no significant effect of seed rate in 2011. The effect of seed rate on PE_N was small compared to the effect of fertilizer N application or year. N regime had a significant effect on PE_N , but the effect was influenced by year, as indicated by a significant interaction. This was a result of the differences in the response of PE_N to delays in fertilizer N application between the years. PE_N for Ntillering was 41.8 kg DM/kg N in 2011 and declined as the first N application was delayed such that PE_N for N37 was 18.2 kg DM/kg N (Table 4). In 2012, PE_N for Ntillering was 22.2 kg DM/kg N, not significantly different from that for N37 in 2011, but significantly lower than that for Ntillering in 2011. In 2012, unlike in 2011, reduction in PE_N as a result of delaying the initial application of N was much lower, with no significant differences detected between the first four N application timings.

Grain N concentration

N regime had a significant effect on grain N concentration, but the effect varied between years (Table 2; Figure 3). Fertilizer addition, irrespective of application timing, significantly increased grain N concentration in both years. Delaying the first application of N resulted in an increase in grain N concentration in both years, but the rate of increase differed between years. In 2011, each additional delay in first N application after GS 30 caused a significant increase in grain N concentration; there was no significant difference between Ntillering and N30. In 2012, the increase in grain N concentration was less pronounced than in 2011 when the first N application was delayed from the tillering stage to GS 32.

Discussion

In terms of the initial application of fertilizer N to winter wheat, this study indicated that, compared to applying fertilizer N at tillering, there was no disadvantage to delaying the first N application until GS 31 in terms of yield. However, as indicated by the significant decrease in yield as the first N application was delayed from GS 30 to GS 31 in 2011, delaying the first N application until after GS 30 can increase the chances of yield loss. These results are in agreement with those of Bodson *et al.* (2001) in Belgium. Schulz *et al.* (2015) also found that delaying N application until the early stem elongation stage had no detrimental effect on yield under high-yielding German conditions.

The absence of a yield reduction as N application was delayed from tillering to GS 31 would indicate that either there was sufficient N in the soil to support the plant's needs during this period or that the plant was able to overcome any temporary deficiency in N that occurred during this period. N deficiency during this early part of the growing season would be most likely to interrupt the tillering process and lead to death of the younger tillers (Masle, 1981, 1985). However, the lack of significant differences in ear population density between the first three N application timings would indicate that, even if tiller death did occur, it was not of sufficient magnitude to reduce the number of ear-bearing tillers surviving to harvest or that

Table 4. Effect of N application timing on recovery (RE_N), agronomic (AE_N) and physiological (PE_N) efficiency of N use

	RE_N^1 (kg N/kg N)	AE_N (kg DM/kg N)		PE_N (kg DM/kg N)	
		2011	2012	2011	2012
Ntillering	60.1 a	25.2 ab	13.3 d	41.8 a	22.2 d
N30	65.8 ab	27.0 a	13.8 cd	40.8 ab	21.2 d
N31	63.7 ab	24.0 b	13.2 d	38.2 b	20.2 d
N32	65.5 ab	20.1 c	12.8 d	29.6 c	20.2 d
N37	69.5 b	13.2 d	9.1 e	18.2 de	13.3 e

¹RE data are the mean of 2011 and 2012.

Means for an efficiency ratio followed by the same letter are not significantly different.

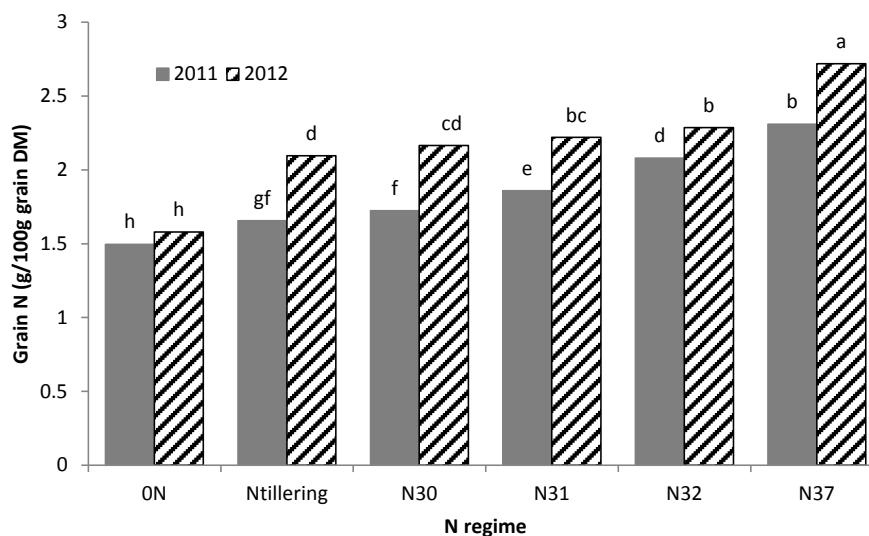


Figure 3. Effect of N regime on grain N concentration of winter wheat (cv. Cordiale) in 2011 and 2012. Bars with the same letter are not significantly different.

compensatory tiller production occurred once N was applied. The ability of the wheat plant to compensate for periods of deficiency early in the season was particularly evident in 2011 when the N37 treatment had the highest ear population density, despite not receiving fertilizer until after the stem elongation phase had been completed. This suggests that even where N is withheld, the crop still retains the ability to compensate by producing additional ear-bearing tillers. However, from a practical point of view, these late-appearing tillers were still immature when the main stem of the same plants had reached maturity, which would cause difficulties for harvesting the crop at the farm level.

Grain yield in wheat is better related to grain number per unit area than to individual grain weight (Slafer and Andrade, 1989). Nitrogen deficiency during the stem elongation phase is more likely to result in reduced GPE by reducing spikelet number or by increasing floret abortion (Langer and Liew, 1973; Whingwiri and Kemp, 1980; McMaster, 1997). There was some evidence that this occurred in 2011 considering that the GPE was reduced when the first application of N fertilizer was withheld until GS 37. This was not evident in 2012, perhaps due to a higher supply of N from the soil, as indicated by the higher N accumulation in samples from areas where no fertilizer N was applied.

The correlation between the reduction in yield encountered as a result of delaying the first N application and a reduction in grain weight in both years, but particularly in 2011, indicated that a restriction on the supply of photosynthate to the developing grain may have been the key effect of delayed N application. Two sources of photosynthate contribute to grain filling in wheat: current photosynthate and photosynthate produced prior to anthesis and stored in the vegetative tissues,

particularly in the stem (Schnyder, 1993). Stem reserves of water-soluble carbohydrate can play an important role in supplying carbohydrate to the grain during grain filling, potentially contributing over two-thirds of the final grain weight in areas where stress conditions occur (Ehdaie *et al.*, 2008). It is possible that stem reserves were diminished where the first application of fertilizer N was progressively delayed due to reduced canopy formation, and hence reduced photosynthesis, during the pre-anthesis stage. However, stem reserves have been found to be higher on a per-stem basis at anthesis in N-deficient wheat than in adequately fertilized wheat, with a corresponding higher grain weight for the N-deficient crop at harvest (van Herwaarden *et al.*, 1998a, 1998b).

A more likely reason could be a reduction in current photosynthesis as a result of reduced leaf area where N application was delayed. Mossedaq and Smith (1994) found that delaying N application until anthesis, compared with applying N just prior to stem extension, caused a reduction in leaf area and therefore reduced assimilation capacity. Additionally, for the N37 treatment, the presence of late-emerging tillers that were harvested before maturity, and therefore, before the grains in those ears had reached their full weight, would have contributed to the lower grain weight in that treatment.

Both experiments in this work were carried out in areas where the risk of take-all caused by *Gaeumannomyces graminis* was low because the previous crop was a non-host of the fungus (Hornby, 1998). Take-all causes a progressive deterioration in root function as the season progresses, which affects nutrient accumulation negatively (Schoeny *et al.*, 2003). This could indicate that delayed N application to crops infected with take-all could lead to reduced capture of that N and consequently reduced yield. Therefore, where take-all is likely to be present,

earlier application of N may be beneficial, although Werker and Gilligan (1990) found no effect of altering N application timing on take-all dynamics.

The increase in RE_N of fertilizer N when the first application was delayed until GS 37, compared to making the first N application at tillering, is consistent with previous findings (Recous and Machet, 1999). However, this increased recovery coincided with a decline in both AE_N and PE_N due to the reduction in yield that occurred when N application was delayed. Therefore, there is a trade-off between RE_N and both AE_N and PE_N . Reducing the amount of N applied at the first application at tillering or early stem extension, and hence when crop demand would have been low, may have increased the RE_N without reducing AE_N or PE_N .

The N application timing treatments in this study were defined by timing of the initial application of N in a two-split approach, with the timing of the second application dictated by the timing of the first. Therefore, the whole fertilizer N regime was shifted in time, not just the initial N application, and it could be argued that the observed treatment effects may have been due, at least in part, to differences in timing of the second application. To avoid confounding the effect of timing of the first application with the timing of the second application, single applications of fertilizer N at the various GSs could have been used. However, studies have shown that applications of high amounts of N before stem extension, as would be the case for the tillering application in this study, can lead to reduced NUE and may increase the risk of lodging and loss of N by leaching (Maidl *et al.*, 1998; Sticksel *et al.*, 2000). In any case, at the farm level, where high amounts of N are being applied, a multiple-split approach is normally recommended (Coulter and Lalor, 2008; Department of the Environment, Food and Rural Affairs, 2010) even though comparisons of single applications with split applications of a given amount of fertilizer N have often shown no effect, positive or negative, on grain yield (Schulz *et al.*, 2015). Therefore, whether the observed effects were due to the timing of the first or second application would be of little concern from a practical point of view.

The non-significant interaction between N regime and seed rate for both yield and ear populations means that there was no evidence found in this study to indicate that crops with a lower plant population required earlier N applications compared to crops with high plant populations. This suggests that there is little scope to manipulate crops with low plant density using the timing of the first N application. Spink *et al.* (2005) also found little evidence to support alterations in the timing of initiation of N fertilization in winter wheat based on plant population despite finding that earlier N application produced more shoots per unit area.

The absence of a significant effect of the pre-sowing slurry application on crop N accumulation in March, before the onset of spring growth, or at anthesis and harvest, where no

fertilizer N was applied, indicates that the nitrogen contained in the slurry was of no net benefit to the crop. Poor recovery by winter cereals of N in autumn-applied slurries has been reported previously as a result of loss of the N from the soil profile during the period of low crop demand over the winter period (Smith and Chambers, 1993; Sieling *et al.*, 1997). This lack of a slurry effect meant that it was not possible to determine objectively whether lower levels of SNS would require earlier applications of fertilizer N, to maintain yield, than situations wherein higher amounts of SNS were present. It is possible that at least some of the differences in the effect of N application timing on yield between the two years were due to differences in SNS. In 2012, when the SNS was higher than in 2011, as indicated by crop accumulation in the absence of fertilizer N, there was no difference in yield as the first N application was delayed from the tillering stage to GS 32. This may indicate that the crop did not experience sufficient levels of deficiency to affect either ear populations or GPE, the two components of yield most likely to be influenced by deficiency during that time. In 2011, when soil N supply was lower, as indicated by crop N accumulation, there was a negative effect on yield when the first N application was delayed until GS 31 compared to the result obtained on making the N application at GS 30. This may have been at least in part due to the higher soil N supply, but the differences cannot be conclusively linked to differences in soil N supply. In particular, the winter period preceding the 2012 growing season was milder and drier than that preceding the 2011 season (Anon, 2011, 2012), which may have resulted in greater amounts of N mineralised and/or less N loss via leaching (Herlihy, 1979; White *et al.*, 1983).

Conclusions

The results of these experiments indicate that, despite greater recovery of the applied fertilizer, in order to avoid yield reductions, the first N application to winter wheat should not be delayed after GS 30. Differences in yield between first N application at tillering, GS 30 and GS31 were dependent on season, with some indication that GS 30 may give the highest yield. Optimum timing of the initial application of fertilizer N was not influenced by plant population density. The work also indicated that autumn slurry application is not an effective strategy to supply N to winter wheat crops under Irish growing conditions.

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