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Meta-analysis of nitrogen fertilization effects on wheat grain protein in Argentina.

Fernando H. Míguez [1]*, Adriana A. Pérez [1], José A. Fresco [1] and María L. Zapiola [1]

[1]*Facultad de Ciencias Agrarias, Universidad Católica Argentina, Freire 183, Buenos Aires, Argentina; Fernando H Míguez ORCID 0000-0002-5086-5884 E-Mails: ferhmiguez@gmail.com; adrianaperez000@gmail.com; jafresco@gmail.com; malu.zapiola@gmail.com*

ABSTRACT

The effect of nitrogen (N) fertilization on yield and grain protein concentration (PC) in wheat was analyzed using a meta-analysis approach. N recovery (NR) was also calculated. We pooled 59 independent field trials containing 171 treatment means conducted on 20 different locations in the Argentinean Pampas from 1995 to 2008. Data was collected from scientific journals, technical reports and published and unpublished studies from the authors. Variables included in the database for each trail were: year, location, ecological zone, variety quality group, timing and rate of N fertilization. For grain yield and PC, treatment means were classified into four groups: early high, early low, late high and late low. For NR analysis, trials were classified into soil-applied N fertilizer, applied up to tillering stage, and foliar-applied N, applied during stem elongation or later. Early soil N fertilization increased yield by 1489 kg ha^{-1} and PC by 0.64% when more than 100 kg N ha^{-1} were applied and by 599 kg ha^{-1} with lower N rates, but had no effect on PC. Late foliar-applied N fertilization increased PC by 1.31% points when the N rate was equal or greater than 20 kg ha^{-1}, but had no effect on yield. Lower N rates increased PC by 0.50% points and slightly increased yield by 270 kg ha^{-1}. Late foliar N fertilization showed a trend for greater NR, averaging 19.01%, than early soil-applied fertilization which averaged 12.71%. Even though a frequent recommendation in Argentina is early N application for increasing PC, here we demonstrate that foliar N fertilization after heading is more likely to increase PC than early soil-applied N.

Keywords: wheat; N fertilization; grain protein concentration.

1. INTRODUCTION

Wheat (*Triticum aestivum L*.) is a major crop in Argentina, where 4.57 million hectares were planted to wheat and production exceeded 15 million metric tons during 2011 [1]. As local consumption accounts for only 5.5 million tons, well over 60% of the wheat produced in Argentina is exported [2]. Main wheat exporter countries, like Canada, Australia and the USA, classify their wheat based on quality for commercialization. However, except for some special contracts for premium quality, Argentinean wheat is commercialized mainly in bulk, without being classified into differential categories regarding milling quality [3, 4]. Improving milling quality through agronomical practices and favoring quality separation could increase Argentinean wheat competitiveness and farmers´ incomes [4, 5].

Grain protein concentration (PC) is one of the basic quality attributes in wheat [6]. Grain PC is usually related to wheat's milling quality and can be modified through fertilization [7, 8, 9]. Grain PC is the result of the balance between carbon (C) and nitrogen (N) grain content and depends on several abiotic and biotic factors, such as drought or heat stress, and insect or fungal infections [6, 10]. Also, grain PC depends on genotype. Therefore, grain PC is the result of the growing environment and genotype interactions.

Growing conditions during grain fill largely affect yield and PC [9]. Wheat crops with the same plant N concentration at anthesis gave different yield and PC when temperature and water availability varied during grain fill [11, 12]. In general, high temperatures reduce the grain fill duration and may reduce the rate of grain filling. Protein concentration may increase under high temperatures due to differential effects of temperature on protein vs. starch deposition [9, 10]. Even though both PC and yield are reduced by high temperatures, the effect is greater on starch accumulation, which depends on the sink strength, than on protein accumulation that depends mainly on N availability [10, 13]. In addition, drought has been found to reduce the duration but not the rate of grain filling in wheat [9, 10]. A reduction in water availability, starting one week previous to anthesis, reduced wheat grain yield but increased PC [8]. Therefore, a stress during grain fill is more likely to result in an increase in PC if there is enough N availability. The potential occurrence of a stress during grain fill makes it difficult to predict crop PC and increases fertilization effect uncertainty. Additionally, the use of fungicides may tend to lower PC by increasing flag leaf green area duration and therefore increasing grain weight, which would dilute PC [14]. In Argentina, wheat planting areas have been classified in seven zones according to their ecological conditions [1]. Wheat is expected to have different yield potential and milling quality in each zone, mainly due to soil quality, water availability and temperature. For a given year and zone, wheat grain milling quality will depend on growing conditions during grain fill and wheat grain could be classified accordingly [5].

Also, wheat quality potential depends on wheat genotype and some wheat varieties are known to produce grain with greater milling quality, regardless of growing conditions. Therefore, genotypes can be classified in quality groups, and varieties within each quality group tend to respond to treatment combinations similarly [8]. However, PC is frequently inversely related to grain yield and high yielding genotypes tend to have a lower PC [7, 15]. Based on germplasm quality potential, Argentinean hard wheat varieties are classified into three quality groups which are: Corrective Wheat or Industrial Panification (group one), Traditional Panification (group two) (+ 8 h fermentation), Direct Panification (group three) (-8 h fermentation) [16].

On top of growing conditions and genotype, N fertilization can further modify PC. The effect of fertilization on milling quality depends on fertilizer type, rate, and timing of application [8]. In addition, the effect of N fertilization on yield and PC varies with N soil availability. When soil N availability is low, N fertilization will mostly increase grain yield and may cause a small increase or even decrease grain PC [17]. When N availability is intermediate, fertilization will most likely increase both yield and PC. Finally, when an N availability threshold is reached, additional N will mostly increase grain PC and have little effect on yield [15, 17]. When base N availability was enough (170 kg N ha^{-1} applied at onset of stem elongation, Zadoks

30) [18], additional N fertilization at second visible node (Zadoks 32) did not affect wheat yield but increased grain PC [8].

Nitrogen plant concentration at flag leaf emergence (Zadoks 39) correlates with PC for a given yield range. Using this correlation, a flag leaf greenness index was developed and is used as a predictor of grain PC [19, 20, 21, 22, 23]. The flag leaf greenness index shows a good fit for predicting PC unless growing conditions during grain fill favor a very high or low yield. Several authors reported that top-dressed N fertilization near crop anthesis increased PC [20, 21, 24, 25, 26]. Application of post-anthesis N, P, and potassium (K) increased protein content of wheat under moderate temperatures by mainly increasing the rate of protein accumulation. However, under a high temperature regime, post-anthesis NPK did not affect the rate or the duration of protein accumulation [27]. Foliar N fertilization is usually more efficient and shows greater N recovery (NR) than soil-applied N, because N is readily absorbed by the leaves and is not exposed to leaching, volatilization or microbiological fixation in soil [28]. In addition, younger plant tissues tend to translocate nutrients more readily to the grains than older leaves [29].

Determining if the effect of timing and rate of N fertilization on wheat grain yield and PC can be generalized over several studies, on different locations, on different years, with different varieties, and sources of N is relevant from a practical point of view, as it could help in defining fertilization strategies aimed at increasing PC on wheat grain in Argentina. Therefore, the aim of this study was to summarize and analyze information available in Argentina on the effect of N fertilization on wheat yield and grain PC. The main objective was to determine if wheat grain PC, and therefore wheat milling quality, can be improved by N fertilization. A secondary objective was to evaluate N use efficiency by calculating the NR for different fertilizer application methods and timing. The main hypothesis is that early soil-applied N fertilization is not reliable for increasing grain PC, while late N fertilization is a useful tool to achieve a greater grain PC, and shows a higher NR.

2. MATERIAL AND METHODS

2.1 Data selection

An extended local literature review was performed and 59 independent field trials containing 171 treatment means on 20 different locations in the Argentinean Pampas from 1995 to 2008 (published up to 2012) were identified to be included in this study (Table 1, Figure 1). Data was collected from scientific journals, technical reports and published and unpublished studies from the authors. Criteria for including the data in the analysis were that trials had to have a standard experimental design showing means, a measure of variability, and sample size for grain yield and PC. Also, all the selected trials presented at least one control plot and one N fertilization treatment. Trials selected for the meta-analysis had wheat varieties from different quality groups.

2.2 Variables and treatment definition

Variables included in the database for each trail were: year, location, ecological zone, variety quality group, timing and rate of N fertilization. For grain yield and PC, treatment means were classified into four groups: early high, early low, late high and late low. Early application included those treatments where N was applied up to flag leaf emergence (Zadoks 39), which in turn were divided into high rate (100 kg N ha^{-1} or over) and low rate (under 100 kg N ha^{-1}). Late application included treatments where N was applied after heading (Zadoks 50) up to start of grain fill (Zadoks 70) and were further divided into high rate (20 kg N ha^{-1} or over) and low rate (under 20 kg N ha^{-1}). Treatments with both N and fungicide applications were not included to avoid confounding effects. The effect of fertilization on wheat grain yield and PC was estimated as the difference between the fertilized and control means.
Regarding NR analysis, trials were classified into soil-applied N fertilizer, which were applied up to tillering stage (Zadoks 29), and foliar-applied N, applied during stem elongation (Zadoks 31) or later. Treatments with both soil and foliar applications were not considered to avoid confounding effects. Nitrogen recovery

was calculated as the amount of N harvested over the control, relative to the amount of N applied. A protein to N conversion factor of 5.75 was used [30]. Therefore, NR represents the percentage of the N applied harvested with the grain.

Table. 1 Description of experiments included in the meta-analysis

				Number of treatments per group				
Year	**Location**	**Zone**	**Variety quality group**	**Early low**	**Early high**	**late low**	**late high**	**Reference**
1995	Venado Tuerto	II North	2	1			2	24
1995	Venado Tuerto	II North	2	1			2	24
1995	Venado Tuerto	II North	2	1			2	24
1995	Venado Tuerto	II North	2	1			2	24
1995	Venado Tuerto	II North	2	1			2	24
1995	Venado Tuerto	II North	2	1			2	24
1995	Venado Tuerto	II North	2	1			2	24
1996	Tres Arroyos	IV	2		1		2	24
1996	Pergamino	II North	2	1			2	24
1996	Pergamino	II North	2	3	2			24
1996	Rojas	II South	2	3	1			24
1997	Balcarce	IV	3		1		2	22
1997	Balcarce	IV	3				2	22
2000	Venado Tuerto	II North	2				2	24
2000	Necochea	IV	2				2	24
2000	Venado Tuerto	II North	2	1			2	24
2000	Rojas	II South	2				2	24
2000	Rojas	II South	2				2	24
2000	Necochea	IV	2				2	24
2000	Necochea	IV	2				2	24
2000	Rojas	II South	2				2	24
2001	Venado Tuerto	II North	2				2	24
2001	Necochea	IV	2				2	24
2001	Necochea	IV	2				2	24
2001	Lobería	IV	2				2	24
2001	N. Olivera	II South	2				2	24
2001	Necochea	IV	2				2	24
2001	Rojas	II South	2				2	24
2001	Necochea	IV	2				2	24

2001	Venado Tuerto	II North	2	1			1	24
2001	Barrow	IV	1	2	1		2	21
2001	Tres Arroyos	IV	1	2			2	31
2001	Barrow	IV	3	2			2	21
2001	Barrow	IV	3	3			2	21
2001	Barrow	IV	2				2	21
2001	Barrow	IV	2	1			2	21
2002	Barrow	IV	1	1			2	26
2002	Barrow	IV	1	1			3	26
2002	Barrow	IV	3		1		2	26
2002	Rafaela	I	2	3			2	32
2002	María Juana	I	2	3			2	32
2004	Balcarce	IV	1		2			20
2004	Mar del Plata	IV	1		1		1	20
2004	Carabelas	II North	2	1		2		33
2004	Gualeguay	III	2	1		1		34
2004	Zárate	II North	2	1		1		35
2004	Puan	IV	2	1		1		36
2004	Rafaela	I	2	1			2	32
2004	Gral Pueyrredón	IV	2				2	37
2004	Balcarce	IV	2		1			37
2005	Balcarce	IV	2		1	1		37
2005	Tandil	IV	2	1			2	37
2005	Rojas	II South	1			2		38
2005	Rafaela	I	2	2		1		34
2005	Rafaela	I	2	2		1		34
2005	San Vicente	I	2	1		1		34
2005	Pergamino	II South	2			1		34
2005	Gualeguay	III	2		1	1		34
2005	Gualeguay	III	2		1	1		34
2005	Tandil	IV	2			2		34
2006	Balcarce	IV	3				2	39
2007	Runciman	II North	2				2	40
2008	Junín	II South	2	3	2	1	1	41

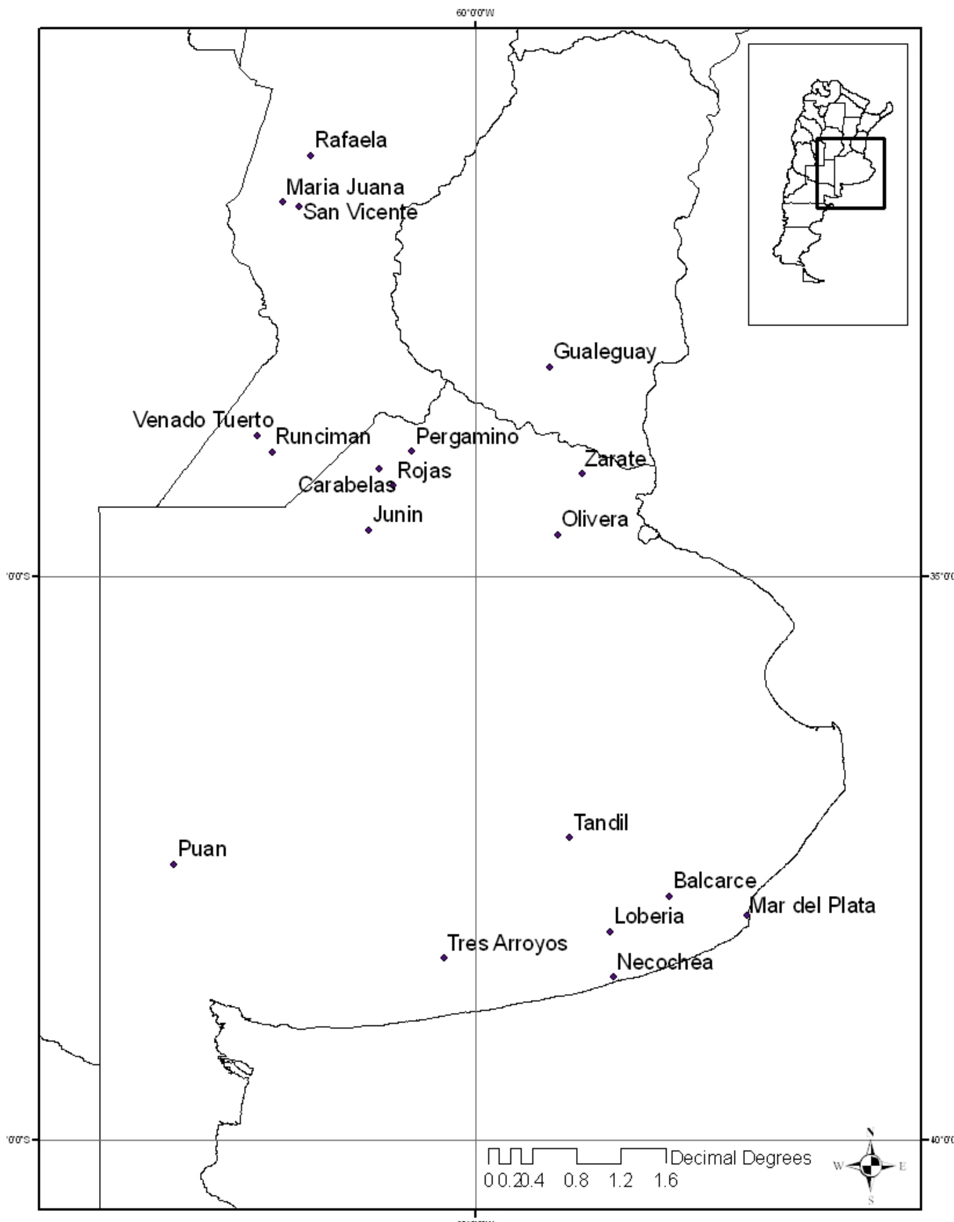

Figure 1 Location of field trials included in the meta-analysis

2.3 Data analysis

The rationale of meta-analysis combined with mixed models was used to analyze the data. As most trials included more than one fertilization treatment, a mixed model was applied with “fertilization treatment” as the fixed effect and “experiment” as the random effect. The meta-analysis approach assumes independence among trials [42]. It was assumed that trials conducted at different locations were independent, even if they were reported in the same publication.

Data were analyzed using restricted maximum likelihood (REML) parameter estimations. All statistical analyses were conducted using the R statistical software, version 2.13.2 (R Development Core Team 2011) with the *lme* package [43]. The Akaike and Schwarz Information Criterion were used to identify the best model. The optimal random effects structure was determined with the likelihood ratio test. Model verification consisted of visually inspecting residuals for normality and homoscedasticity. An effect was considered significant at an $\alpha = .05$ if the 95% confidence interval (CI) did not overlap a value of 0.

3. RESULTS AND DISCUSSION

3.1 N fertilization effect on yield and PC

There was a 1489 kg ha^{-1} (95% CI: 963 to 2016) increase in yield when over 100 kg N ha^{-1} were applied, while a smaller increase 599 kg ha^{-1} (95% CI: 393 to 805) was observed when the N rate was under 100 kg ha^{-1} (Figure 2). Average controls yield was 4002 kg ha^{-1}, so these increases represent a 37.21% and 14.96% yield increase over controls for early high and early low rate N fertilization, respectively. However, early season soil-applied N had no effect on PC when rate was under 100 kg N ha^{-1} (early low 0.09%, 95% CI: -0.14 to 0.32) (Figure 3). When N rate was higher there was a slight PC increase (early high 0.64%, 95% CI: 0.16 to 1.12). A field trial [41] showed a higher increase in PC as a response to early soil-applied fertilization, but growing conditions during reproductive stages were unusually warm and dry in that experiment, determining a 50% yield reduction of the control in relation to the expected yield based on genotype and production environment. Therefore, this trial was considered an outlier and was not included in the PC analysis.

These results partially contradict a usual local recommendation that N fertilization at tillering can be useful to increase grain PC [44, 45, 46, 47], as the observed effect is small. Most soils where wheat is grown in the Argentinean Pampas have low N availability; therefore soil N fertilization tends to increase yields and has little effect on grain PC. Wheat export taxes and N fertilizer/wheat price relationships in Argentina induce farmers to apply low fertilizer rates, and local research has been conducted with N rates within that range. No fertilization treatment exceeded 200 kg N ha^{-1} among the analyzed experiments. Therefore, even the treatments classified as early high may not have had enough N as to increase both yield and PC, and are not very high if we compare them to the N rates applied routinely in other wheat-growing countries.

Late season top-dressed fertilization slightly increased yield (270 kg ha^{-1} 95% CI: 34 to 507) when N rates were under 20 kg ha^{-1} (Figure 2). Late high N fertilization did not increase yield (45 kg ha^{-1}, 95% CI: -65 to 155). Even though some treatments showed a trend for yield reduction, no phytotoxic effects were reported in any experiment and we did not observe any fertilizer toxicity in our experiments.

Late season foliar N fertilization increased PC by 1.31% points (95% CI: 1.14 to 1.49) when the N fertilizer rate was equal or greater than 20 kg ha^{-1} and had a slight effect (0.50% points 95% CI: 0.17 to 0.82) when the N rate was lower (Figure 3). Average PC for the control was 10.94%. Therefore, the observed increase in PC represents a 12% and 4.6% increase for late high and late low N fertilization practices, respectively.

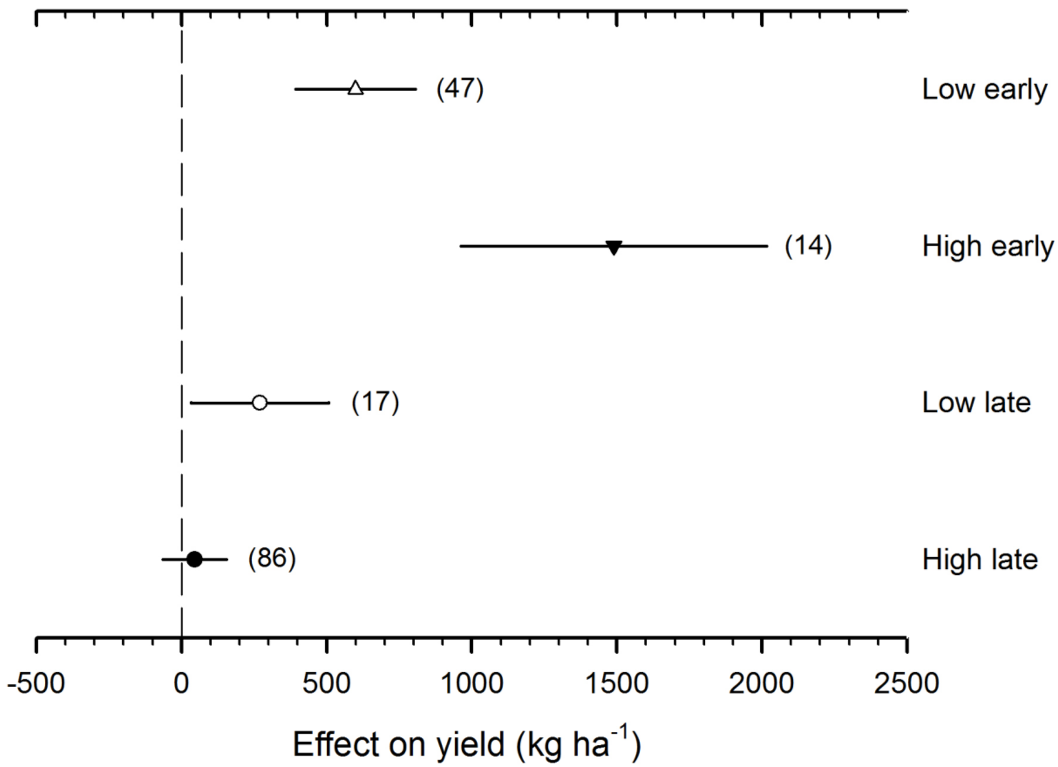

Figure 2. Fertilization effect on yield according to N application rate and timing. Means, 95% confidence intervals and number of cases (in parenthesis) for each treatment are shown.

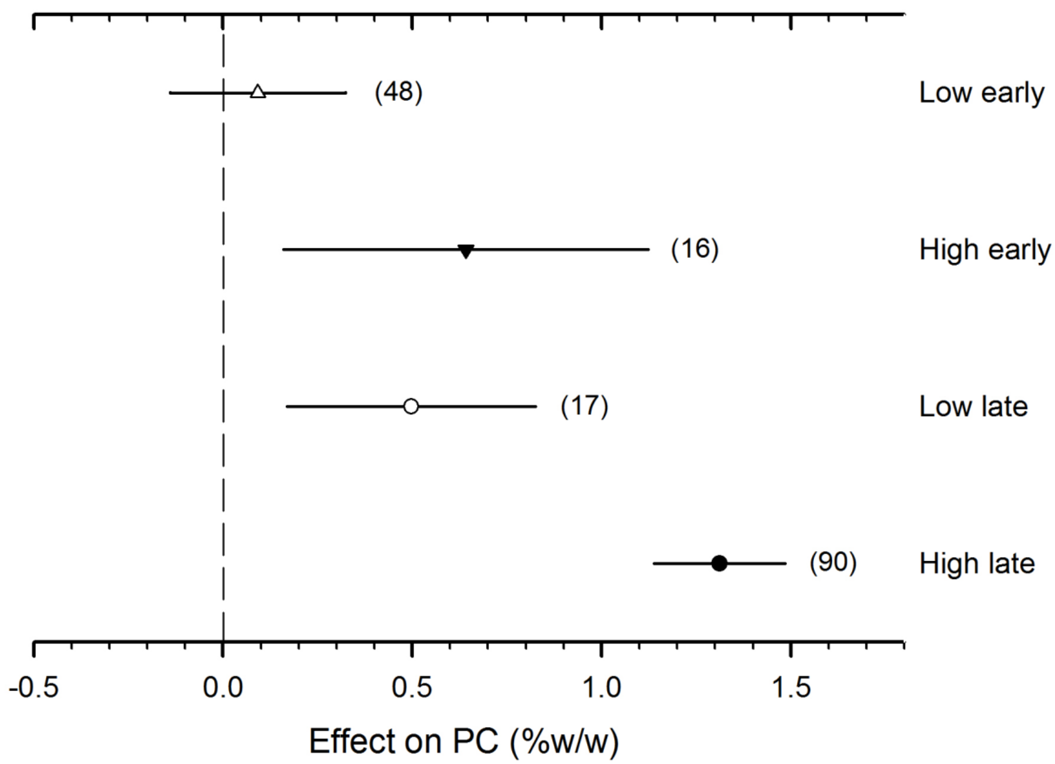

Figure 3. Fertilization effect on PC according to N application rate and timing. Means, 95% confidence intervals and number of cases (in parenthesis) for each treatment are shown.

Wheat quality from different production areas were analyzed for three growing seasons by Correa and Lerner 2003 [5], who estimated that Argentinean wheat would have obtained a 55% increase, no change, and a 15% discount on the export price for top, medium, and low quality, respectively. Therefore, grain separation according to wheat grain quality in Argentina could have resulted in a substantial income increase for farmers in the analyzed growing seasons. With the present commercialization system, a 1% increase in PC results only in a 2% increase in price. In Argentina, a large proportion of the crop harvest is stored on the farms in large plastic bags that contain about 200 tons each. The milling quality potential and the actual grain quality could be assessed on the farm to separate the grain using NIRS protein meters in the combines and/or by conducting a pre-harvest PC determination [48]. This onsite separation could be useful to sell the grain according to its milling quality instead of mixing the grain in storage facilities and selling an average quality product. Therefore, fertilization management could improve wheat grain quality, but grain separation according to milling quality at harvest time is necessary to result in greater profits for farmers, as separation would allow them to sell wheat according to market requirements [3, 4].

3.2 N recovery

There was no difference in NR for N application timing. However, early soil-applied N fertilization had a trend towards lower NR values averaging 12.71% (95% CI -14.33 to 39.75) than late foliar N fertilization with an average NR of 19.01% (95% CI 3.28 to 34.74) (Figure 4). Also, foliar application showed greater variability in NR. Some trials had a NR greater than 100%, probably due to an augmented grain set which increased the sink strength and N absorption or remobilization from the rest of the plant. The greatest NR was associated with those experiments where a very low N rate was applied, implying a small dividing factor. Some field trials showed a negative NR, most likely due to a slight decrease in yield for the treatment in comparison to the control.

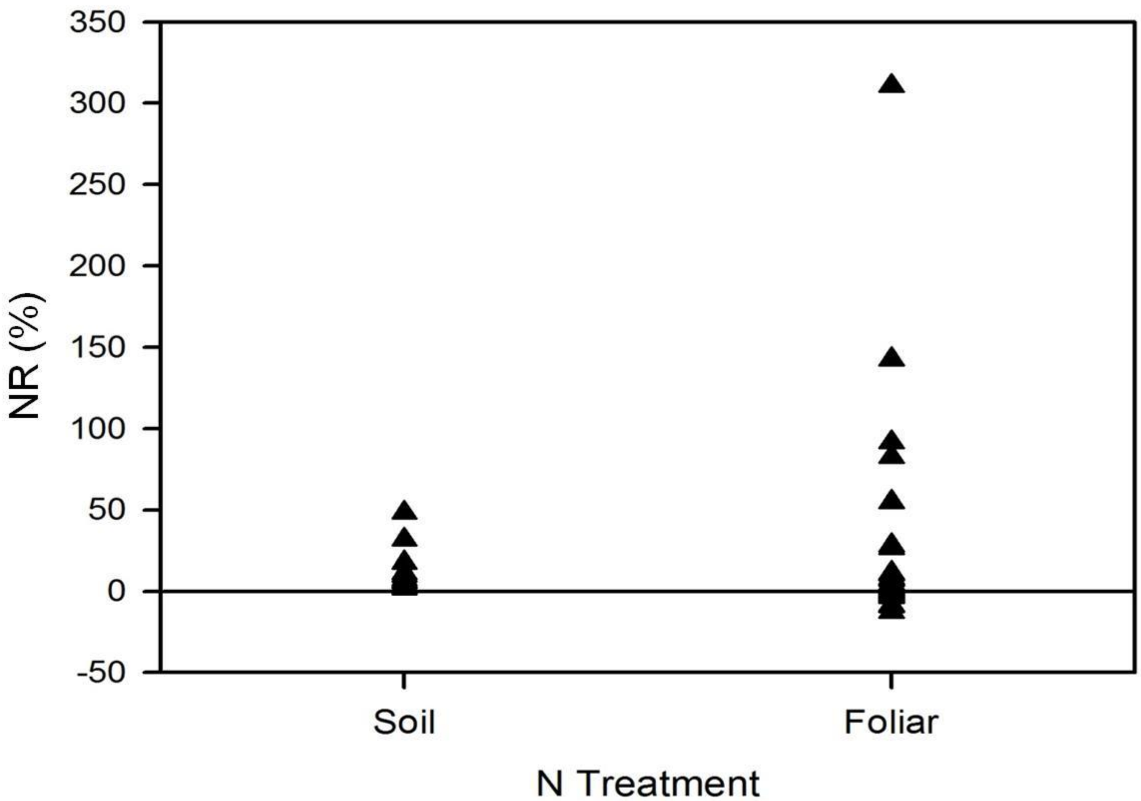

Figure 4. Nitrogen recovery for soil and foliar N application.

4. CONCLUSION

Timing and rate of N fertilization had a different effect on grain yield and PC in the Argentinean Pampas. If the aim of N fertilization is to increase PC in wheat grain, over 20 kg N ha^{-1} should be applied after heading. However, the potential effect will depend greatly on growing conditions during grain fill. Although soil-applied early-season N fertilization increased grain yield, it does not seem to be a sound method to increase PC in wheat in the Argentinean Pampas. Finally, foliar fertilization showed a trend to greater NR but also greater variability in NR than soil- applied N fertilization.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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