Cover crop species can increase or decrease the fertilizer-nitrogen requirement in maize

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Core Ideas

Calculations suggest that a cover crop of hairy vetch + oat increased the N requirement.

Calculations suggest that a cover crop of hairy vetch reduced the N requirement.

Delaying the termination of high grass mixture cover crops reduced maize yields.

The chlorophyll meter at silking could assess the N status in maize.

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ABSTRACT

Cover crop species and termination date could affect fertilizer-N management and N diagnostic methods traditionally used in bare fallow-maize (*Zea mays* L.) systems. Our objectives were to (a) assess the impact of cover crop termination date on maize yield and response to fertilizer-N, (b) determine maize yield at varying N rates following different cover crop species and mixtures, and (c) evaluate the chlorophyll meter reading (CMR), grain N concentration (Nc), and grain N nutrition index (NNI) as N diagnostic methods. Experiments were conducted at four sites in the southeastern Argentinean Pampas with maize planted after cover crops. Factors investigated were cover crops with vetch (*Vicia villosa* Roth), vetch-oat (*Avena sativa* L.) mixture, or bare fallow; early termination of cover crops or about 3 wk later; and maize fertilizer-N at 0, 50, 100, and 200 kg N ha^{-1}. The minimum N rate that maximized grain yield was higher in vetch-oat mixture (100 kg N ha^{-1}) than in vetch (50 kg N ha^{-1}). Maize yield was not affected by cover crops with 200 kg N ha^{-1}. Late termination dates of vetch-oat mixture reduced maize yield by 15% compared with early termination dates, while there was no effect of vetch termination date. Cover crop termination date did not affect yield response to fertilizer-N. Relative CMR at silking, grain N_C, and especially grain NNI at maturity were useful tools to diagnose maize N status and cover crop effect. Cover crop management should be considered to adjust the fertilizer N rate and optimize maize productivity.

Abbreviations: CMR, Chlorophyll meter reading; F, Fallow; HV, Hairy vetch; HV+O, Hairy vetch-oat mixture; Nc, Nitrogen concentration; NNI, Nitrogen nutrition index.

1. INTRODUCTION

Agricultural intensification arises to partially satisfy a growing food demand by maximizing crop productivity per unit area with a minimal environmental impact. In this context, cover crops could assist with the goal of intensification through their contributions to multiple ecosystem services (Dabney et al., 2001; Daryanto et al., 2018). Cover crops play a key role in N management in agricultural systems by modifying the N dynamic in soil. For example, grasses species reduced N losses during the fallow period (Martínez et al., 2013; Restovich et al., 2012). Legume species incorporate N into the system by biological fixation (Dabney et al., 2001). Also, after cover crops are terminated, and during biomass decomposition, N supply could increase or decrease in the soil for the following crop in the rotation, depending on the species (Murungu et al., 2010).

Nitrogen commonly limits maize (*Zea mays* L.) yield, and most prior research on yield response to fertilizer-N was during a time that cover crops were not considered. The N release rate from the residues of cover crops will depend on the carbon (C) to N ratio (C:N) of those tissues, mainly affected by the species and its maturity at the time of termination (Jahanzad et al., 2016; Sievers & Cook, 2018). For example, Sievers and Cook (2018) reported for a single termination date a rapid N mineralization from the legume relative to the grass, leading to a possible asynchrony between crop N demand and residue mineralization. Therefore, mixtures of legumes and grasses could improve the aforementioned synchrony. Regarding the time of termination, late termination dates of grasses immobilized more N relative to early termination dates (Roberts, 2020). In legumes, late termination dates increased N accumulation in the cover crop and improved N supply for the subsequent maize crop (Clark et al., 1997). Hence, the termination date of the cover crop will affect not only N supply but also the N response of the following crop in the rotation, but this effect can differ due to the amount and type of residues. The interactions between termination dates and different cover crops species [hairy vetch

(HV, *Vicia villosa* Roth) and HV plus oat (*Avena sativa* L.) mixtures (HV+O)] on the response to fertilizer-N in maize is a critical research topic that warrants further investigation.

The evaluation of N diagnostic methods is crucial to define the accurate fertilizer-N rate and maximize maize production while limiting environmental and economic costs. Chlorophyll meter reading (CMR) is one of the most widespread and non-destructive methods to assess maize N status (Barbieri et al., 2013; Sainz Rozas et al., 2019; Zhao et al., 2018). For diagnostic purposes, the relative CMR (CMRr) index should be calculated as a ratio between the CMR from the area of interest and the CMR from an area that is not N-limited (Barbieri et al., 2013; Pagani et al., 2009). The CMRr has been proposed as an accurate maize N diagnostic method but has not been evaluated in maize following cover crops.

Grain nutrient concentration has been suggested as a tool to diagnose maize nutrient status (Carciochi et al., 2019; Sutradhar et al., 2017). Although grain analysis does not allow in-season N deficiency adjustments, this information can guide nutrient management recommendations for the following crop in the rotation (Smith & Loneragan, 1997). Barbieri et al. (2013) reported a critical threshold for maize grain N concentration (Nc) of 10.8 g kg^{-1}. However, this critical value needs to be validated for maize growing after different cover crop species.

Another relevant tool for diagnosing N status is the N nutrition index (NNI) concept, expressed as the ratio of the actual plant Nc and the critical Nc at the same biomass level (Plénet & Lemaire, 1999). The critical Nc is the minimum Nc in biomass that ensures maximum crop growth. Critical Nc varies with the biomass level and could be calculated from critical N dilution curves, which relate both variables (i.e., critical Nc vs. biomass). Critical N dilution curve and NNI were first developed for maize aboveground biomass in vegetative stages (Plénet & Lemaire, 1999) and later calibrated for maize ear organ from silking to physiological maturity stage, using grain Nc and grain biomass (yield) at this last stage (Zhao et al., 2020). As NNI accounts for variations on both biomass and Nc, it could be more accurate than just considering the organ Nc. Thus, NNI is proposed as a proper N diagnostic tool for identifying changes in nutrient use efficiency across management systems (Lemaire & Ciampitti, 2020).

In this study, we hypothesize that: (a) N needs for maize varies when using cover crops relative to the fallow, and with different cover crop species and termination dates, (b) CMR at silking provides an adequate in-season N status of maize following cover crops, and (c) NNI at maturity is a more accurate N diagnostic method than the grain Nc (without taking into account differences in yield). Therefore, our objectives were to: (a) assess the impact of cover crop termination date on maize yield and its response to fertilizer-N, (b) determine maize yield at varying fertilizer N rates following different cover crop species and mixtures, and (c) evaluate and compare the CMRr (at silking), grain Nc, and NNI (grain, at maturity) as N diagnostic methods.

2. MATERIALS AND METHODS

2.1. Site Description and Experimental Design

Rainfed field experiments were conducted during the 2019-2020 season at four sites (S1 to S4) in the southeastern Argentinean Pampas (37-39 °S, 58-60 °W) on Typic Argiudoll soils (USDA, 2014) (Table 1). The regional climate is Cfb (temperate, no dry season, warm summer), according to the Köppen classification (Kottek et al., 2006). The average temperature and rainfall in the area during the cover crop and maize growing season are 12.2 ºC and 629 mm, and 18.6 ºC and 462 mm, respectively. Terrain elevation was 147 m at S1, 197 m at S2, 86 m at S3, and 99 m above sea level at S4. A no-till system was used at all sites for at least the previous 15 years, and sunflower (*Helianthus annuus* L.) (S1 and S2) or barley (*Hordeum vulgare* L.) (S3 and S4) were the previous crops. Soils were characterized by high organic matter contents (> 47 g kg^{-1}) and loam to silt clay loam textures (Table 2). Additional information on soil characteristics is presented in Table 2.

The experimental design at each site was a randomized complete block design with a split-plot arrangement (S1) or split-split plot arrangement (S2, S3, and S4) with three replications (plot size 3 or 4 m x 10 m). At S1, the main plot treatments were hairy vetch (HV), hairy vetch plus oat (HV+O), and no cover crop (fallow; F); and sub-plot treatments were 0, 50, 100, and 200 kg N ha^{-1} applied to maize. At S2, S3, and S4, the main plot treatments were HV, HV+O, and F; the sub-plot treatments were early (~33 days before maize sowing) and late (~11 days before maize sowing) cover crop termination date. The sub-sub-plot treatments at S2 were 0, 50, 100, and 200 kg N ha^{-1} applied to maize, while at S3 and S4 were 0 and 200 kg N ha^{-1} applied to maize. Urea (46% N) was the N fertilizer source. Additionally, 30 kg P ha^{-1} as triple superphosphate (20% P) and 20 kg S ha^{-1} as gypsum (18% S) were applied to all plots to avoid P and S deficiency, respectively. All fertilizers were surface broadcast after maize sowing, representing the fertilizer practices typically used by farmers in the area.

Cover crops were sown between middle March and April (Table 1) at 0.21 m row spacing, using a seeding rate of 20 kg ha^{-1} for HV and, in the mixture, 30 kg ha^{-1} of HV plus 10 kg ha^{-1} of oat. The HV was inoculated with *Rhizobium leguminosarum var. viciae,* and all cover crops were fertilized at sowing with 12.6 kg N ha^{-1} and 14 kg P ha^{-1} as diammonium phosphate. The F treatment was kept free of weeds by chemical control. Cover crops were chemically terminated using 1 to 2 kg a.i. ha^{-1} of glyphosate [N-(phosphonomethyl) glycine] from the end of September to early November (Table 1). Maize was sown from the end of October to the end of November, depending on the site (Table 1), with a 0.52 m row spacing at S1, S2, and S4, and 0.70 m at S3. Additional information on the cover crops and maize management is presented in Table 1.

For all sites, rainfall and daily mean temperature data were obtained from meteorological stations located in the experimental sites. The rainfall data were reported for the cover crop growing season, cover crop termination date to maize sowing, and maize growing season.

2.2. Soil and Plant Analysis

Soil samples were taken at maize sowing (0 to 20-cm depth) in the F plots to characterize each site. The samples were dried at 30 ºC and ground to pass a 2-mm sieve. Organic matter (Walkley & Black, 1934), pH (1:2.5 soil/water ratio), texture (Bouyoucos, 1962), and N mineralized in short-term anaerobic incubation (Nan) (Keeney, 1982) were determined. Also, soil samples were taken at 0 to 20 and 20 to 60-cm depths in each sub-plot, and soil NO_3^--N concentration was quantified colorimetrically (Keeney & Nelson, 1982). Nitrate N content was calculated using a bulk density estimated as proposed by Hollis et al. (2012).

The cover crop shoot biomass was quantified in each block by cutting plants at ground level (0.25 m^2) before termination. Plant samples were dried (60 ºC), weighed, and ground (1-mm sieve), and N concentration was determined by Kjeldahl method. Nitrogen uptake in cover crop shoot biomass (Table 1) was calculated as the product of N concentration and dry weight biomass. Also, the carbon (C) to N ratio (C:N) was estimated using a C concentration of 440 g kg^{-1} (Duval et al., 2017).

At maize silking (R1; Abendroth et al., 2011), CMR was recorded using the N-tester chlorophyll meter (Yara, Dülmen, Germany). Readings were taken at the midpoint of the uppermost fully developed leaf in 30 plants from the middle rows of each plot. After physiological maturity, five linear meters from the two central rows were hand-harvested and threshed using a stationary threshing machine. Grain yield was adjusted at 145 g kg^{-1} water content. Furthermore, 1000-grain weight was determined by counting and weighing 200 grains, and the number of grains m^{-2} was calculated from grain yield and 1000-grain weight. The grain Nc was determined using a near-infrared spectroscopy instrument TEC-NIR 256 (TecnoCientífica, Buenos Aires, Argentina), after validating calibrations with the Kjeldahl method.

2.3. Calculations

The grain yield response to fertilizer-N was calculated for each site, termination date, and cover crop as the difference between yield with 200 kg N ha^{-1} and yield in the unfertilized control (0 kg N ha^{-1}). The CMRr was calculated as the ratio of the CMR measured for a given cover crop-N rate to the CMR of the HV with 200 kg N ha^{-1}, considered as the non-limited N treatment. Likewise, the relative grain yield was calculated as the ratio of the grain yield obtained for a specific treatment to the yield of the HV with 200 kg N ha^{-1}.

The N nutrition index (NNI) was computed as the ratio between grain N_C and the critical Nc (critical N_C) (Equation 1). The critical N_C was calculated using grain biomass critical N_C curve for maize (Zhao et al., 2020) (Equation 2).

$\mathrm{NNI} = \frac{\mathrm{grain\ Nc}}{\mathrm{critical\ Nc}}$ (Equation 1)

$\mathrm{citical\ Nc} = \mathrm{a_c}\ (\mathrm{W})^{-\mathrm{b}}$ (Equation 2) where $a_c = 22.2$ (g kg^{-1}); W= grain biomass (Mg ha^{-1}); b = 0.26

2.4. Statistical Analysis

Different analyses of variance (ANOVA) were conducted using the R software (R Core Team, 2018). Four-way ANOVA was performed for the analysis of grain yield, grain number, 1000-grain weight, CMR, and grain Nc, where site, cover crop, termination date, and N rate were the evaluated factors. Also, a three-way ANOVA was performed to assess the effect of site, cover crop, and termination date on maize yield without fertilizer-N and response to fertilizer-N. Due to multiple interactions in all the variables evaluated, we presented only the interactions related to our experimental objectives. The complete descriptive database is presented in Supplemental Tables S1 and S2. The normality of the data was tested via the Shapiro-Wilks test, and the homogeneity of variances was evaluated and confirmed by the Levene-test ($p>0.05$) (R Core Team, 2018). Means were compared by the Tukey test ($p<0.05$).

The relationships between relative grain yield and CMRr or grain NNI and CMRr were fitted using the *lm* procedure included in the R software (R Core Team, 2018). Relative grain yield was expressed as a function of grain Nc and grain NNI through a linear-plateau model: $y = a + b * x$ if $x \leq c$ and $y = a + b * c$ if $x > c$, where “a” is the intercept, “b” is the slope during the linear phase, and “c” is the value of x at which the linear model reaches a plateau.

3. RESULTS

3.1. Weather conditions

Total rainfall ranged from 225 to 390 mm for the cover crop growing season, 0 to 141 mm between cover crop termination and maize sowing, and 436 to 562 mm during the maize season (Figure 1). Rainfall between cover crop termination to maize sowing was ~65 mm more with early termination than late termination. Considering the rainfall from the cover crop termination date to the maize harvest, it fluctuated from 481 to 659 mm. Only at S1 and $S3_L$, the rainfall was lower than the crop water demand (~550 mm), which may have limited maize attainable yields. The average daily mean temperature for each environment and period (cover crop and maize growing seasons) was similar to the historical records and did not negatively affect crop growth (data not shown).

3.2. Cover crop characteristics

The average shoot biomass for HV was 2033 kg ha^{-1} for early and 4507 kg ha^{-1} for late termination date, and for HV+O it was 3839 kg ha^{-1} for early and 7021 kg ha^{-1} for late termination (Table 1). Nitrogen uptake ranged from 25 to 143 kg N ha^{-1} in HV and from 37 to 143 kg N ha^{-1} in HV+O. The N uptake was on average 59 and 35 kg N ha^{-1} higher with late than early termination dates for HV and HV+O, respectively. Regarding cover crop residue quality, the C:N ratio for HV was 15:1 and 18:1 and for HV+O was 28:1 and 31:1 for early and late termination dates, respectively.

3.3. Grain yield and yield components

Neither four-way nor three-way interactions were observed on grain yield (Table 3). However, N rate effect on grain yield depended on the cover crop treatment and vice versa, among other two-way interactions less relevant for our objectives. Grain yield ranged from 4185 to 14045 kg ha^{-1} (Supplemental Table S1). The lowest yields were observed with HV+O and the highest yields with HV (Figure 2). Fertilizer-N increased yield in all the cover crop conditions. The minimum N rate that statistically maximized grain yield was higher in HV+O (100 kg N ha^{-1}) than in

HV (50 kg N ha^{-1}). Without fertilizer-N, HV+O produced the lowest yield (6428 kg ha^{-1}) and F and HV the highest (7548 and 8504 kg ha^{-1}, respectively). There was no difference in yield among cover crops with 200 kg N ha^{-1} (avg. 10060 kg ha^{-1}). Grain number and grain yield had similar responses to N, but 1000-grain weight only increased with fertilizer-N in some situations, mainly with HV+O (Supplemental Table S1). Therefore, maize yield (kg ha^{-1}) variability was mainly explained by changes in grain number (grains m^{-2}) ($y=1007+2.4x$; $R^2=0.86$; $p<0.05$) rather than 1000-grain weight (g) ($p=0.37$).

3.4. Cover crop termination date

No site x cover crop x termination date interaction was observed on maize yield without fertilizer-N (Table 4). However, the termination date effect on maize yield varied within cover crops. On average among sites, the late termination date of HV+O reduced maize yield by 15% (1109 kg ha^{-1}), and there was no effect of HV termination date on maize yield.

Yield response to fertilizer-N was not affected by cover crop termination date (Table 4). However, yield response to fertilizer-N varied between cover crops, with the response after HV+O (2692 kg ha^{-1}) being greater than after HV (1835 kg ha^{-1}).

3.5. Nitrogen diagnostic methods

Three-way interactions (site x cover crop x termination date and site x termination date x N rate) were observed on CMR (Table 3). As it was not our intention to analyze treatment effects on CMR but to use this variable as a diagnostic method, deep analyses on interactions were not performed. Overall, the difference in CMR between the maximum N rate (200 kg N ha^{-1}) and the control (0 kg N ha^{-1}) followed the order HV+O (114) > F (73) > HV (61) (Supplemental Table S2). Thus, the CMRr calculated for the control was 91% for HV, 86% for F, and 81% for HV+O.

When the dataset was pooled, a positive linear relationship between the relative grain yield and the CMRr was observed (Figure 3a).

A four-way interaction was observed (site x cover crop x termination date x N rate) on grain Nc (Table 3). Overall, at 0 kg N ha^{-1}, grain Nc was 10.4 g kg^{-1} for HV, 9.9 g kg^{-1} for F, and 9.6 g kg^{-1} for HV+O (Supplemental Table S2). Interestingly, grain Nc increment due to fertilizer-N (i.e., the difference in grain Nc between the maximum N rate and the control) decreased from 2.3 g kg^{-1} with early to 1.2 g kg^{-1} with late termination date in HV and similarly for HV+O from 2.5 g kg^{-1} to 1.7 g kg^{-1}.

Linear-plateau models represented the relationship between relative grain yield and grain Nc (Figure 3b) or NNI (Figure 3c). Thus, a grain Nc lower than 11.9 g kg^{-1} and a grain NNI value below 0.95 indicated reductions in relative grain yield and, consequently, N deficiency. While, for grain NNI and CMRr, a positive linear relationship was observed (Figure 3d).

4. DISCUSSION

Maize yields increased with HV relative to fallow, confirming other results for cereal summer crops (Carciochi et al., 2021; Frasier et al., 2017; Pott et al., 2021). However, maize yield was reduced with HV+O mixture compared with the fallow, which contradicted the yield increases for legume-grass cover crops reported by Clark et al. (1997), Miguez and Bollero (2005), and Restovich et al. (2012). In legume-grass mixtures, as the grass proportion increases in the mixture, less N is available due to a greater immobilization (Frasier et al., 2017; Sainju et al., 2006), impacting N supply for the following crop in the rotation and its yield (Restovich et al., 2012). This immobilization caused by cover crop mixtures with grasses was observed in our study, where the higher C:N ratio of HV+O (avg. 30:1) reduced soil

N availability at maize sowing (avg. ~38 kg N ha^{-1}) compared with HV (avg. ~59 kg N ha^{-1}) with a 16:1 C:N ratio.

The mineralization-immobilization turnover caused by the residues from different cover crop species was reflected in the maize yield without fertilizer-N. Compared with F, we observed an overall yield increase due to HV of 960 kg ha^{-1} and yield reduction due to HV+O of 1120 kg ha^{-1}, compared with F. Therefore, considering a N requirement of ~18 kg N Mg grain^{-1} (Ciampitti & Vyn, 2012; Setiyono et al., 2010) and a N uptake efficiency of 0.55 kg kg^{-1} (Ladha et al., 2005), it could be deduced a mineral fertilizer-N saving of 31 kg N ha^{-1} with HV and -37 kg N ha^{-1} with HV+O. Even though it is known that HV could provide other benefits to the crops rather than only N, no differences in grain yield were observed among cover crops with 200 kg N ha^{-1}. This suggests that N availability was the main factor affected by cover crops, and thus, it controlled maize yield.

Yield response to fertilizer-N was greater with HV+O, mainly explained by a greater N immobilization and a slower N release rate in the mixture, compared with HV (Ranells & Wagger, 1996). The low or null response to N with HV could be associated with the low C:N ratio and the ability to supply significant N as the legume incorporates N into the system via the biological N fixation mechanism (Vaughan & Evanylo, 1998). Enrico et al. (2020) reported that HV fixed on average 99 kg N ha^{-1}, increasing N availability and reducing the N fertilizer required by maize. Contrarily to our results, Utomo et al. (1990) and Vaughan et al. (2000) reported that maize planted after HV increased yield but did not reduce the need for N fertilizer. This fact was possibly caused by (a) early termination dates of the cover crop, with greater asynchrony between N supply and crop demand, and (b) a high yield environment, increasing crop N demand. Further studies should investigate the dynamics between the N release from cover crops residues and N demand by maize crop for this complex cover crops-maize systems.

Cover crops termination date influences N dynamic during the maize growing season (Clark et al., 1997; Parr et al., 2011). The C:N ratio in grasses increases with plant maturity (Greenwood et al., 1990). Thus, in those legume-grass mixtures with high grass component, if the termination date is delayed, it would negatively impact maize yield. Thus, as it was reported by Vaughan and Evanylo (1998) and validated by our results, late termination dates of HV+O reduced maize yield. For legume cover crops, as the termination date is delayed, more N will be potentially fixed (Enrico et al., 2020), allowing to attain high yields (Clark et al., 1997). However, we did not observe any effect of HV termination date on maize grain yield. The high soil organic matter content and consequently N supply via mineralization (Nan) in the evaluated sites, which agree with the values typically observed in the studied area (Reussi Calvo et al., 2014), potentially reduced the yield response to N fertilization. Thus, the extra N accumulated in HV with late termination dates was not translated into higher maize yield.

Plant-based diagnostic methods of N deficiency are valuable tools to understand N dynamic and improve its management under cover crop-maize systems (Carciochi et al., 2021). Previous studies stated that CMRr was an accurate N diagnostic method in maize growing after fallow (Barbieri et al., 2013; Sainz Rozas et al., 2019). Our results demonstrated that CMRr determined at silking is also a valuable tool for characterizing in-season changes in maize N status originated by cover crops, termination dates, and N rates.

We observed that grain Nc explained variations in relative grain yield, and the explanation capacity was improved when grain biomass was also considered (i.e., through grain NNI calculation). This is a valuable indicator to understand N use efficiency traits by its independence on plant size (Lemaire & Ciampitti, 2020). In our study, a threshold of 0.95 was found for grain NNI at maturity, similar to the one reported by Ziadi et al. (2008) (0.93) at V_{12} stage in shoot biomass and slightly above that observed by Fernandez et al. (2020) (0.88) at silking in shoot biomass. Specifically for maize following HV, Pott et al. (2021) found NNI thresholds of 1.1, 1.35, and 1.4 for determinations done in shoot biomass at silking for low, medium, and high-yield environments, respectively. Finally, to characterize the NNI through a non-destructive method, we explored the association between CMRr at silking with grain NNI at maturity. As reported by Zhao et al. (2018) for V_6 to V_{12} growth stages, the mentioned relationship indicates that CMRr is a useful in-season diagnostic tool that reflects variations in NNI, which is a more accurate N diagnostic method.

5. CONCLUSION

Our study demonstrates that the selection of cover crop species (hairy vetch vs. hairy vetch + oat) and termination date (only in hairy vetch + oat) affect maize yield. Additionally, cover crop species differentially impacted the potential savings in fertilizer-N. Therefore, cover crop species should be considered to define the fertilizer-N need for maize. From the diagnostic aspect, chlorophyll meter readings at silking and especially grain NNI at maturity were useful tools to diagnose the crop N status, assisting with a more integral N management for the cover crop-maize rotation. Future studies should be focused on understanding the impact of cover crops management practices on the synchrony between N supply from cover crops and N demand from maize and its effect on grain yield and the response to fertilizer-N.

CONFLICT OF INTEREST

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

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FIGURE CAPTIONS

Figure 1. Rainfall (in mm) during the cover crop growing season, from cover crop termination date to maize sowing, and during the maize growing season in seven environments (four sites S1 to S4 with early (subscript E) and late (subscript L) cover crop termination date).

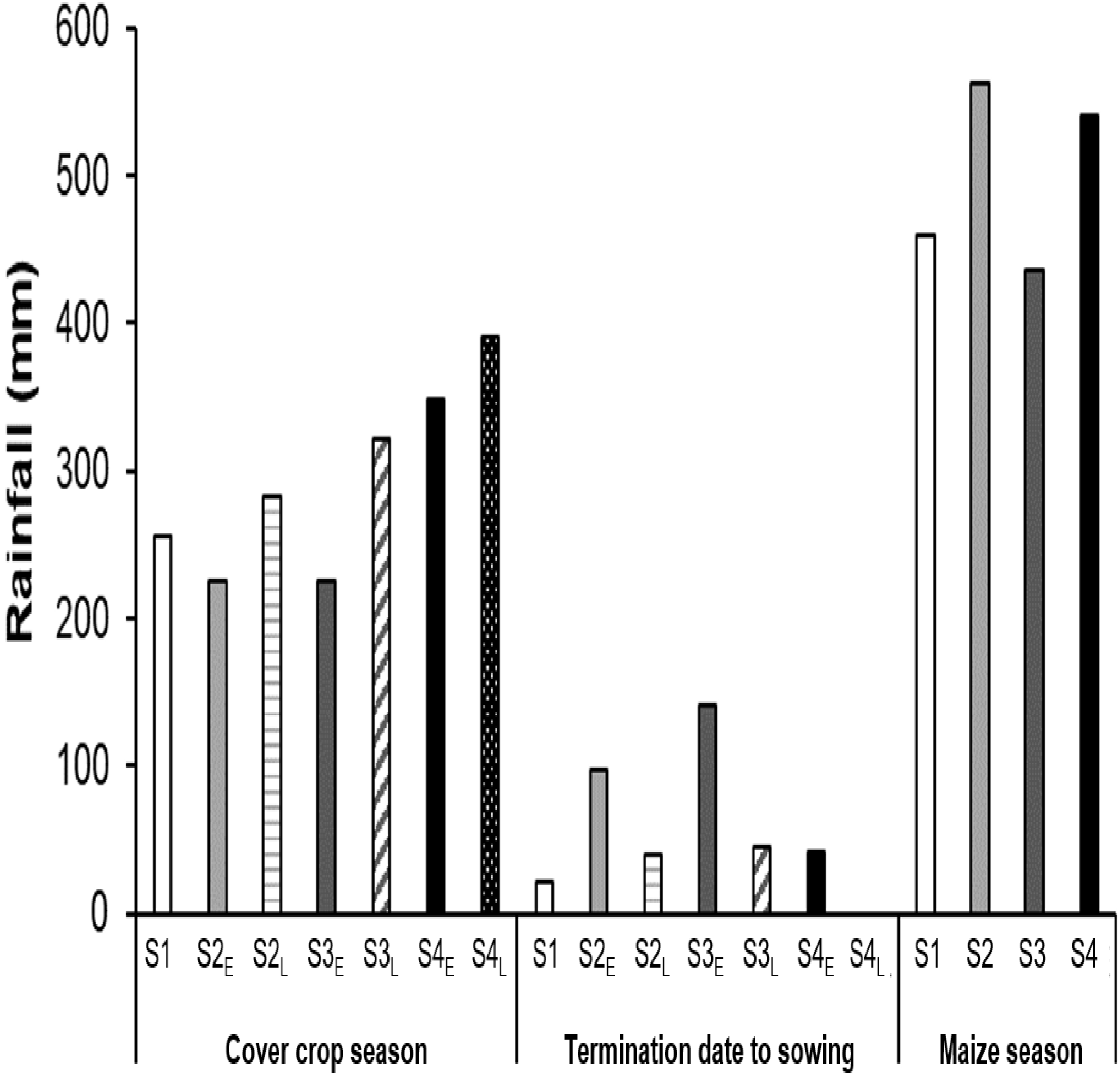


Figure 2. Maize grain yield (kg ha^{-1}) growing after different cover crops (F, fallow; HV, hairy vetch; HV+O, hairy vetch-oat mixture) and nitrogen (N) rates (N0 = 0, N50 = 50, N100 = 100, and N200 = 200 kg N ha^{-1}) at four sites in Argentina in 2019-2020. Means followed by the same lowercase letter within a cover crop and by the same capital letter within a N rate are not significantly different by Tukey test at p<0.05. Vertical bar in each column indicates the standard error of the mean.

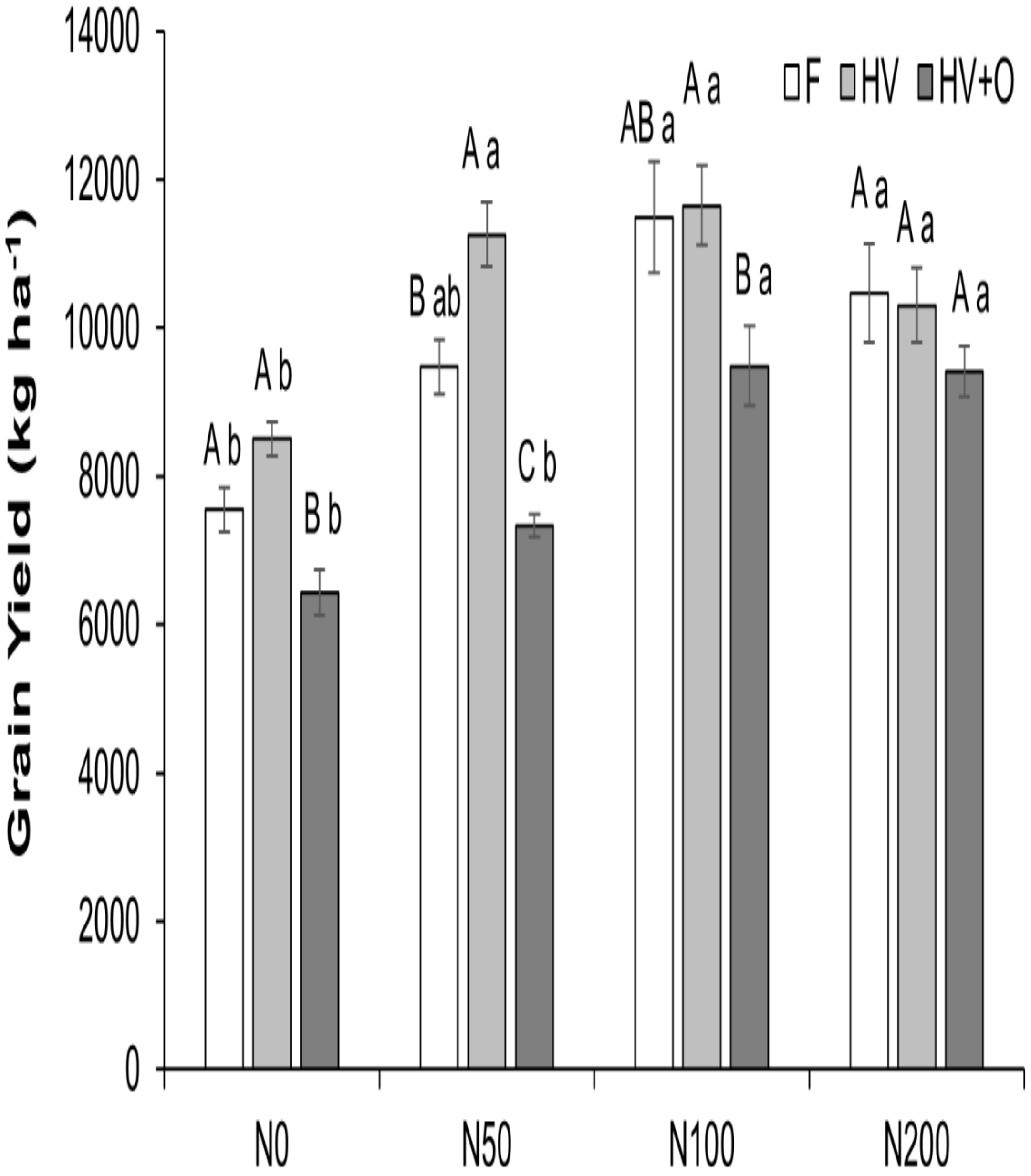
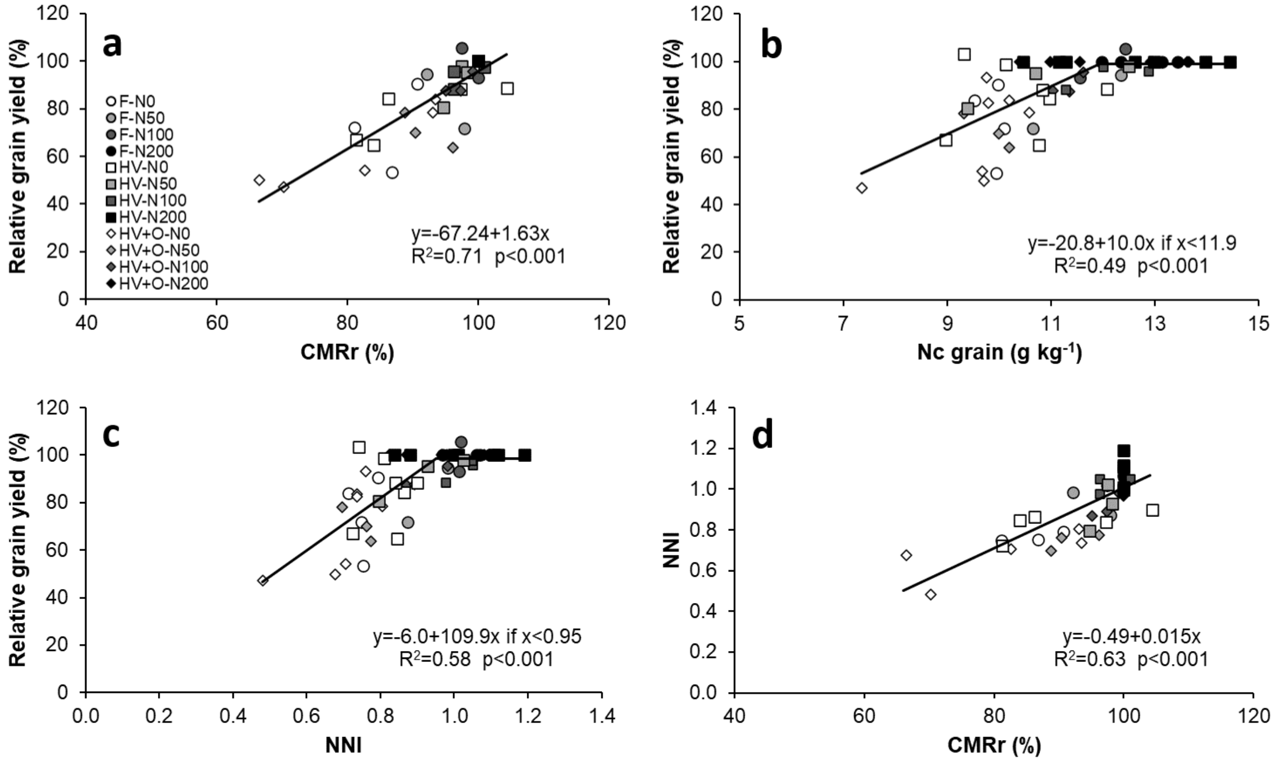


Figure 3. Relationship between maize relative grain yield (%) and (a) relative chlorophyll meter reading (CMRr) at silking, (b) nitrogen concentration (Nc) in grain, and (c) grain nitrogen nutrition index (NNI) calculated at maturity, and between grain NNI and CMRr (d) in experiments combining four nitrogen rates (N0, N50, N100, and N200) with three previous conditions [fallow (F), hairy vetch (HV), and hairy vetch + oat (HV+O)]. The relative grain yield was calculated as the ratio of the grain yield obtained for a specific treatment to the yield of the HV with 200 kg N ha^{-1}. The CMRr was calculated as the ratio of the CMR obtained for a specific treatment to the CMR of the HV with 200 kg N ha^{-1}. Experiments were conducted at four sites in Argentina in 2019-2020.



TABLES

Table 1. Location, cover crop management and characteristics, and maize management in cover crops-maize experiments conducted at four sites in Argentina in 2019-2020.

Characteristics	Site			
	S1	**S2**	**S3**	**S4**

Location				
Lat.	-37.6068	-37.0801	-38.2152	-38.2943
Long.	-58.6439	-59.5892	-59.0170	-59.0167
Cover crop management and characteristics				
Sowing date	23-Apr	18-Apr	12-Apr	15-Mar
Termination date [a]				
Early	16-Oct	22-Sept	5-Oct	30-Oct
Late	-	12-Oct	5-Nov	14-Nov
Shoot biomass, kg ha^{-1}				
HV [b]				
Early	3960	1450	850	3800
Late	-	2970	5150	5400
HV+O				
Early	5740	1916	2600	7000
Late	-	4850	6480	9733
N uptake, kg ha^{-1}				
HV				
Early	112	46	25	93
Late	-	91	105	143
HV+O				
Early	83	30	37	132
Late	-	85	76	143
C:N				
HV				
Early	16	14	15	16
Late		14	22	17
HV+O				
Early	31	29	31	26
Late		25	38	30
Maize management				
Sowing date	1-Nov	24-Oct	29-Nov	15-Nov
Plant density, pl ha^{-1}	51400	51000	40000	32000
Hybrid	DK-7210	Nidera AX 7761	Nidera AX 852	Next 22.6

[a] Early (~33 days before maize sowing); Late (~11 days before maize sowing).

[b] HV, hairy vetch; HV+O, hairy vetch-oat mixture.

Table 2. Soil characterization [soil type, texture, organic matter (OM), pH, N mineralized in short-term anaerobic incubation (Nan), and NO_3^--N] at maize sowing in cover crops-maize experiments conducted at four sites in Argentina in 2019-2020.

Site	Soil type	Textural class [a]	OM [a]	pH [a]	Nan [a]	NO_3^--N [b]				
						F [c]	HV		HV+O	
							Early [d]	Late	Early	Late
			g kg^{-1}		mg kg^{-1}	----- kg ha^{-1} -----				
S1	Typic Argiudoll	Loam	53.	5.	53.	97.	86.	-	37.	-

			5	7	1	2	5		7	
S2	Typic Argiudoll	Loam	64.9	6.0	60.3	58.2	74.9	91.2	48.1	48.8
S3	Petrocalcil Paleudoll	Sandy Clay Loam	47.1	6.0	54.1	58.7	49.9	39.8	64.0	27.3
S4	Petrocalcil Paleudoll	Sandy Clay Loam	58.3	6.2	63.9	47.5	37.8	35.3	17.7	21.2

[a] 0-20 cm.

[b] 0-60 cm.

[c] F, fallow; HV, hairy vetch; HV+O, hairy vetch-oat mixture

[d] cover crop termination date: Early, ~33 days before maize sowing; Late, ~11 days before maize sowing.

Table 3. ANOVA for the effect of site, cover crop, termination date, N rate, and their interactions on grain yield, grain number m^{-2}, 1000-grain weight, grain nitrogen concentration (Nc), and chlorophyll meter reading (CMR).

Source of variation	Grain yield	Grain number m^{-2}	1000-grain weight	Grain Nc	CMR
Site (S)	***	***	***	***	**
Cover crop (CC)	***	ns	***	ns	ns
Termination date (TD)	***	**	ns	*	**
N rate (N)	***	***	***	***	***
S x CC	***	**	***	ns	ns
S x TD	***	ns	***	ns	*
S x N	***	*	***	**	***
CC x TD	***	ns	***	ns	ns
CC x N	**	*	**	ns	*
TD x N	ns	*	ns	ns	ns
S x CC x TD	ns	ns	*	ns	***
S x CC x N	ns	*	ns	ns	ns
S x TD x N	ns	ns	ns	ns	*
CC x TD x N	ns	ns	ns	ns	ns
S x CC x TD x N	ns	**	ns	*	ns

*, **, ***, significant at the 0.05, 0.01, and 0.001 probability level, respectively.

NS, nonsignificant.

Table 4. Mean ± standard error across sites and ANOVA F-test probabilities for maize grain yield without fertilizer-N and yield response to fertilizer-N with 200

kg N ha^{-1} (N response) in experiments testing two cover crops and two termination dates at three sites in Argentina in 2019-2020.

Cover crop [a]	Termination date [b]	Grain yield	N response
		kg ha^{-1}	kg ha^{-1}
HV	Early	8492 ± 222 a [c]	1799 ± 763
	Late	8477 ± 481 a	1872 ± 805
HV+O	Early	7356 ± 307 b	2674 ± 798
	Late	6247 ± 345 c	2710 ± 738
		ANOVA	
Source of variation			
Site (S)		*	***
Cover crop (CC)		***	**
Termination date (TD)		**	ns
S x CC		**	ns
S x TD		ns	ns
CC x TD		**	ns
S x CC x TD		ns	ns

[a] HV, hairy vetch; HV+O, hairy vetch-oat mixture.

[b] Early, ~33 days before maize sowing; Late, ~11 days before maize sowing.

[c] Within columns, means followed by the same letter are not significantly different according to Tukey test ($p<0.05$). The lack of letters in a comparison indicates no differences between termination dates.

, **, *, significant at the 0.05, 0.01, and 0.001 probability level, respectively.

ns, nonsignificant.