

# Wheat improves nitrogen use efficiency of maize and soybean-based cropping systems

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24 **Abstract**

25 Integrated nitrogen (N) management strategies could make significant  
26 contributions to improving the efficiency of N use in the northern Corn Belt,  
27 particularly for maize, which has high N requirements. Using legume cover crops  
28 has been shown to increase both the soil's capacity to supply N, and Nitrogen Use  
29 Efficiency (NUE), through the reduction in the amount of N fertilizer that must be  
30 applied to the following crops. However, the impact of non-legume crops such as  
31 winter wheat (*Triticum aestivum* L.) on the diminishing return function between  
32 crop yield and N supply and its influence on N fertilizer use remains unclear. We  
33 hypothesized that maintaining wheat in short maize and soybean- based rotations  
34 is instrumental to improve cropping system performance and increase N fertilizer  
35 use efficiency while decreasing N requirements for maize. Seven maize and  
36 soybean rotations with different frequency of winter wheat with or without  
37 underseeded red clover (*Trifolium pratense* L.) were grown in two tillage systems  
38 (conventional and zone-tillage) and four long-term N regimes in Ridgetown, ON,  
39 Canada (2009-2013). Wheat in the rotation increased maize and soybean yields,  
40 negated crop yield lags due to zone-tillage, and decreased maximum economic  
41 rates of fertilizer N (MERN). The benefits of wheat in the rotation on maize yield  
42 were negated by high N rates; however, similar yields were obtained with lower N  
43 levels in rotationally grown maize, resulting in a 17% (conventional till) to 21%  
44 (zone-till) increase in Partial Factor Productivity for N fertilizer at MERN (PFP<sub>MERN</sub>).  
45 While N benefits to crops following wheat alone may be attributed to a higher  
46 indigenous plant available soil N, underseeding red clover further increased the

47 agronomic efficiency (AE) of N fertilizer ( $AE_{MERN}$ ) up to 32%. Maize yields were  
48 also less limited by N supply and less responsive to N fertilization when grown in  
49 rotation with wheat, especially in the zone-till system. These results highlight the  
50 value of wheat as a system component of dominant maize/soybean short rotations  
51 of Ontario and its potential to increase both maize and soybean productivity using  
52 less N input.

53

54 **Keywords:** nitrogen, wheat, maize, soybean, nitrogen use efficiency, MERN,  
55 rotation diversity.

## 56 **1. Introduction**

57 Over the past several decades, crop diversity in the northern Corn Belt (Ontario  
58 and North Central US) has substantially declined and rotations consisting solely of  
59 maize and/or soybean increasingly dominate the landscape (Figure 1). Increases  
60 in maize and soybean acreage has corresponded with reductions in acreages of  
61 grasslands, forages and other small cereal grains (Figure 1) (Liebman et al., 2013;  
62 Nickerson et al., 2007; Wright and Wimberly, 2013).

63 Agronomic and environmental consequences of declining rotation diversity have  
64 been well documented. Loss of rotation diversity has been associated with  
65 reductions in soil organic matter, aggregate stability and soil quality (Dapaah and  
66 Vyn, 1998; Havlin et al., 1990; Katsvairo et al., 2002; McDaniel et al., 2014;  
67 Munkholm et al., 2013; Raimbault and Vyn, 1991; Van Eerd et al., 2014; Varvel,  
68 1994), increased soil erosion (Langdale et al., 1991; Rachman et al., 2003; Tisdall  
69 and Oades, 1982), increased greenhouse gas emissions (Drury et al., 2008; Liebig  
70 et al., 2005; Meyer Aurich et al., 2006), decrease in yield potential and increased  
71 yield instability (Grover et al., 2009; Katsvairo and Cox, 2000; Lund et al., 1993;  
72 Meyer-Aurich et al., 2006; Singer and Cox, 1998; Smith et al., 2008; Stanger and  
73 Lauer, 2008; Varvel, 2000; Yamoah et al., 1998a).

74 Many of the agronomic and environmental consequences associated with losses  
75 of crop rotation diversity also influence soil nitrogen (N) processes, N losses and  
76 crop response to N (Culman et al., 2013; Havlin et al., 1990; McDaniel et al., 2014;  
77 Shipitalo et al., 2013; Stecker et al., 1995; Varvel and Peterson, 1990). For  
78 instance, there is considerable evidence that removal of legumes, such as alfalfa

79 (*Medicago sativa* L.), red clover (*Trifolium pratense* L.) or soybean (*Glycine max*  
80 (L.) Merr.) from a maize (*Zea mays* L.) based rotation increase optimum N  
81 fertilization rates and have a significant impact on N dynamics (Bruulsema and  
82 Christie, 1987; Gentry et al., 2013; Henry et al., 2010; Hesterman et al., 1992;  
83 Liebman et al., 2012; Stecker et al., 1995; Stute and Posner, 1995; Wivstad, 1999).  
84 Furthermore, increasing N fertilization has also been shown to decrease rotational  
85 benefits of legumes on maize yields in various studies (Adams et al., 1970;  
86 Copeland and Crookston, 1992; Crookston et al., 1991; Nevens and Reheul, 2001;  
87 Peterson and Varvel, 1989; Porter et al., 1997; Riedell et al., 1998; Singer and  
88 Cox, 1998; Stecker et al., 1995). However, much less is known when non-legume  
89 species, such as winter wheat (*Triticum aestivum* L.), are removed from common  
90 maize-based rotations in the northern Corn Belt.

91 The potential effect of rotation diversity on crop response to N fertilization is of  
92 interest given escalating N fertilizer costs (USDA-NASS, 2014a) and continuing  
93 concerns about the negative impact of fertilizer N production and potential losses  
94 on environmental quality (Lebender et al., 2014; Peoples et al., 2004; Syswerda et  
95 al., 2012). Increasing N Use Efficiency (NUE) has also been a long-lasting  
96 research goal, particularly for maize, which is a major user of N. Although the  
97 amount of maize grain produced per unit of N applied ( $PFP_N$ ) in the United States  
98 has increased linearly by 36% in the last 21 years (from 42 kg kg<sup>-1</sup> in 1980 to 57  
99 kg kg<sup>-1</sup> in 2000), due to a combination of high yielding hybrids and improvement  
100 in crop management, the amount of N fertilizer recovered in aboveground plant  
101 biomass during the growing season ( $RE_N$ ) remains relatively low (~37% across

102 various rotations in the North-Central USA (Cassman et al., 2002), and significant  
103 opportunities remain to improve N fertilizer use practices in maize. For instance,  
104 the impact of wheat in maize-soybean rotations on the diminishing return function  
105 between maize yield and N supply and NUE is not well understood.

106 We hypothesized that maintaining crop rotation diversity is instrumental to increase  
107 productivity, maize N fertilizer use efficiency and decrease crop N requirements.  
108 We used yield data (2009-2013) gathered at a long-term N regime and rotation  
109 trial to quantify benefits of maintaining wheat in short maize- and soybean-based  
110 rotations on: 1) cropping system's productivity, 2) crop N requirements, 3) NUE,  
111 and 4) whether the temporal niche provided by winter wheat for red clover or tillage  
112 system influences these responses.

113

## 114 **2. Material and Methods**

### 115 *2.1. Study site*

116 Research was conducted from 2009 to 2013 on a field trial that was established at  
117 the University of Guelph Ridgetown Campus, Ridgetown, ON (42°26'N, 81°53'W)  
118 in 1995. The soil was an Orthic Humic Gleysol clay loam with 1.6% to 2.3% organic  
119 carbon in the top 15 cm in 2009 (Van Eerd et al., 2014). Weather data were  
120 recorded on-site at ~200 m from the experiment; data included hourly air  
121 temperature at 1.25 m above the soil surface and daily rainfall. Average monthly  
122 temperature and precipitation for the last decade (2003-2013) are shown in Figure  
123 2A. Deviations from monthly long-term averages for both temperature and  
124 precipitation during the study are presented in Figure 2 B,C.

125            *2.2 Experimental design and treatments*

126    Since 1995, treatments were established annually on the same plots and were  
127    arranged as a split-split plot design with four replications. The treatments were  
128    tillage system on the main plot, crop rotation on the split-plot and N rates on the  
129    split-split-plot.

130    The main-plot treatment consisted of two tillage systems: conventional and zone-  
131    till for maize. For maize and soybean, conventional tillage consisted of moldboard  
132    plowing in the fall at a depth of 0.20 m, followed by two or three passes with a field  
133    cultivator in the spring at a depth between 0.07 to 0.08 m. Prior to 2012, the zone-  
134    till treatment for maize consisted of two planter-mounted coulters per row. In 2012,  
135    the zone-till maize treatment was modified by tilling zones in the fall using a Trans-  
136    Till (Row-tech, Snover, MI, USA) since very few local maize growers use no-till  
137    practices on similar fine-textured soils because of unfavorable seedbeds or delays  
138    in maize planting (Vyn and Hooker, 2002). Maize was then planted into these tilled  
139    zones in the spring with the same planter equipped with two coulters per row. In  
140    all years of the long-term trial for maize, the inter-row spaces were left  
141    undisturbed.

142    From 1995 to 2008, the split-plot consisted of five crop rotation regimes:  
143    continuous maize (MM), continuous soybean (SS), maize-soybean (MS),  
144    soybean-winter wheat (SW) and maize-soybean-winter wheat (MSW). In 2009, a  
145    red clover treatment was introduced by frost seeding into the wheat stand in March  
146    of every year in all wheat plots by splitting across the width. This resulted in two  
147    additional rotation treatments from 2009-2013: SW with the wheat underseeded to

148 red clover (SWrc) and MSW with the wheat underseeded to red cover (MSWrc).  
149 Crop rotations with more than one crop were duplicated or triplicated so that all  
150 crops within each rotation were present in every year. Impact of the red clover split  
151 implemented in 2009 on crop yields could be first measured in 2011 for soybean  
152 (SW vs SWrc) and 2012 for maize (MSW vs MSWrc). Since no significant effects  
153 of red clover history could be detected in 2010 or across study years (Figure 3),  
154 yield data from 2010 and onward were used for all rotations.

155 The split-split-plot treatment consisted of four N rates in the maize and wheat. No  
156 N fertilizer was applied to soybean. Each maize and wheat split-plot (6.1-m wide  
157 x 24-m long) was divided along the length to represent the four N rate treatments,  
158 which were kept consistent across the whole duration of the rotation since the  
159 experiment was established in 1995. From 2009 to 2013, total N rates for maize  
160 were 12, 72, 132, and 192 kg N ha<sup>-1</sup>, and 0, 50, 100, and 150 kg ha<sup>-1</sup> for wheat. In  
161 maize, N treatments consisted of 12 kg N ha<sup>-1</sup> applied as a starter fertilizer (150 kg  
162 ha<sup>-1</sup> of 8-32-16) on all treatments, with the balance of N sidedress-applied as urea  
163 ammonium nitrate (28-0-0) at about the V3 developmental stage. The UAN was  
164 knifed-in or injected at 10 cm deep between crop rows. In winter wheat, 100 kg ha<sup>-1</sup>  
165 of monoammonium phosphate (11-52-0 or MAP) was applied at planting,  
166 followed by urea (46-0-0) or ammonium nitrate (33-0-0) at Zadoks 21 up to the  
167 target N rate. All N rates used in the analysis include the total N applied in both the  
168 starter and in-crop applications and were not adjusted when red clover was  
169 included in the rotation. From 1995 to 2008, N rates ranged from 0 to 150 kg N ha<sup>-1</sup>  
170 and 0 to 120 kg N ha<sup>-1</sup> in maize and wheat respectively.

171            *2.3 Crop management*

172    The cultivars planted along with planting and harvesting dates are presented for  
173    each crop in Table A.1. From 2009 to 2013, maize was seeded at 84,000 seeds  
174    ha<sup>-1</sup> in 0.76 m-wide rows with a 4-row no-till planter (John Deere 7000, Moline, IL).  
175    Soybean was seeded at 400,000 seeds ha<sup>-1</sup> in 0.38 m-wide rows with an eight-  
176    row-unit no-till planter (Kearney Planters Inc., Thamesville, ON). Wheat was  
177    seeded at 1,600,000 seeds ha<sup>-1</sup> and single-cut red clover was frost-seeded at 10  
178    kg ha<sup>-1</sup> in early March. The same maize, soybean, and wheat cultivars were never  
179    planted for more than two successive years throughout the study (Table A.1), and  
180    they were chosen according to their popularity among local growers. Weeds were  
181    controlled in maize and soybean with both pre- and post-emergent herbicides. In  
182    wheat, post-emergent herbicides were applied when needed. Plots were  
183    maintained so that pests and weed pressure did not differ between treatments and  
184    that productivity was not adversely affected by those factors.

185    The middle two rows of each four-row-plot were harvested for yield determinations  
186    in maize, and a 1.5-m wide swath was harvested from the middle rows of each  
187    soybean and wheat plot. In all years of the experiment, crop residues were  
188    returned to the plot area after the grain was harvested. Grain yields, grain  
189    moistures, and test weights were measured on plot combines equipped with  
190    HarvestMaster GrainGage Classic grain measurement systems (Juniper Systems,  
191    Inc., Logan, UT). Red clover plant population densities were estimated visually in  
192    September between 2009 and 2013.

193            *2.4 Crop response to Nitrogen*

194 Regression analyses were performed using treatment means across years to  
195 estimate maize and winter wheat grain yields at increasing N rates from 2010 to  
196 2013. Grain yields were not estimated beyond 192 kg N ha<sup>-1</sup> in maize and 150 kg  
197 N ha<sup>-1</sup> in wheat.

198 The impact of N fertilization on crop-specific rotational benefits to maize yields was  
199 measured by fitting regression models to the estimated delta ( $\Delta$ ) yield between two  
200 treatments (Table 2). N<sub>max</sub> was calculated from regression equations, and was  
201 defined as the N rate at which grain yields (N<sub>max<sub>Y</sub></sub>) or rotational effects (N<sub>max<sub>r</sub></sub>)  
202 was maximized for each treatment.

203 The Maximum Economic Rate of Nitrogen (MERN, kg N ha<sup>-1</sup>) was defined as the  
204 N rate that produced maximum return to N investments and Maximum Economic  
205 Yield (MEY, kg grain yield ha<sup>-1</sup>). MERN was calculated from quadratic regression  
206 equations describing the maize yield responses to fertilizer N (Gaudin et al., 2014;  
207 Rajsic and Weersink, 2008; Vyn et al., 2000):

208 [1]  $MERN = [b - (F/P)] / 2c$

209 where b and c are linear and quadratic coefficients from the yield response  
210 equations (yield = a + bN + cN<sup>2</sup>), F is the cost of fertilizer N (CAN\$ kg<sup>-1</sup>) and P is  
211 the price of maize (CAN\$ kg<sup>-1</sup>). The average farm value of maize and cost of urea  
212 fertilizer in Ontario from 08/2009 to 08/2013 were used (CAN\$0.22 kg<sup>-1</sup> of maize  
213 at farm value and CAN\$1.54 kg<sup>-1</sup> of N as urea, OMAFRA 2014).

214 Partial Factor Productivity for N fertilizer at economic optimums (PFP<sub>MERN</sub>, ,  
215 Cassman et al., 2002) represents yields obtained per unit of N applied at MERN  
216 for each rotation and tillage treatments (PFP<sub>MERN</sub> = MEY/MERN). Agronomic

217 Efficiencies of N fertilizer at MERN ( $AE_{MERN}$ , Cassman et al., 2003) account for  
218 differential plant available soil N of the different treatments in NUE calculation:

219 [2]  $AE_{MERN} \text{ (kg kg N}^{-1}\text{)} = ((MEY - Y_{N=0})/MERN)$

220 where  $Y_{N=0}$  is the yield intercept (a) of the N response curves. Incremental  
221 Agronomic Efficiencies of N fertilizer ( $AE_i$ , Cassman et al., 2003) estimate yield  
222 gains obtained per unit of incremental N rate:

223 [3]  $AE_i \text{ (kg kg N}^{-1}\text{)} = dY/dF = (Yield_N - Yield_{N-1})/(N \text{ rate}_N - N \text{ rate}_{N-1})$

## 224 *2.5 Statistical Methods*

225 Statistical analyses were performed using SAS statistical software (Statistical  
226 Analysis System, version 9.3, SAS institute, NC, USA). Residuals were found  
227 homogeneous, normal-distributed using the Shapiro–Wilk W test ( $P=0.98$ ) and no  
228 significant outliers were detected by Lund’s test. Mixed models were used for  
229 analysis of variance, with crop rotation, tillage system and N as fixed effects, and  
230 year and replication as random effects. PROC NLIN with Marquardt iterative  
231 method was used to fit crop yield response to N rate to quadratic plateau models.  
232 Models were constrained such that the linear regression coefficient was greater  
233 than or equal to 0, and the quadratic coefficient was less or equal to 0 (Gaudin et  
234 al, 2014). Comparison of predicted values from regression curves were based on  
235 a t-test using standard errors obtained from the Mixed model. Type I error rate was  
236 set at 0.05 for all tests.

## 237 **3. Results**

### 238 *3.1 Winter wheat in rotation increases maize and soybean yields*

239 Inclusion of winter wheat in the crop rotation increased maize and soybean yields,  
240 but the magnitude of impact depended on the tillage system in both crops (Table  
241 1). Crop rotation with winter wheat increased soybean yields by 0.61 and 0.32 Mg  
242 ha<sup>-1</sup> in tilled and zone-tilled systems, respectively ( $P<0.05$ ; Figure 4). Inclusion of  
243 wheat also eliminated the 6% soybean yield lag in the tilled treatments compared  
244 to zone-till observed in the SS and MS rotations (Figure 4).

245 Compared to soybean, the maize yield response to crop rotation and tillage system  
246 was dependent on the year ( $P<0.001$ ; Table 1) and variations in monthly  
247 precipitation and temperature among growing seasons (Figure 2). Abnormally wet  
248 and cool spring conditions in 2011 (Figure 2) delayed planting, which probably was  
249 the main reason for lower maize yields and the lack of crop rotation effects in both  
250 tillage treatments compared to other years (Figure 3). In 2012, abnormally dry  
251 conditions and above normal temperatures during vegetative growth (Figure 1)  
252 appeared to negate crop rotation effects in the tilled systems (Figure 3). However,  
253 in the zone-till system, the inclusion of winter wheat increased maize yields by  
254 18.8% in the dry year (Figure 3). In the two other years of the study (2010 and  
255 2013), maize yielded the lowest in the MM and MS crop rotations in the tilled  
256 system (Figure 3). Despite different year environments, maize response to  
257 treatment did not interact with year (Table 1, Figure 3). On average, winter wheat  
258 improved maize performance by 16.6% and 18.8% in the zone-till and till systems,  
259 respectively, and negated yield reductions due to MM in the zone-till system  
260 (Figure 3). Our results show no significant rotation and tillage effects on wheat  
261 yields, which remained stable across both treatments (Table 1).

262           3.2 *Wheat rotation benefits are N dependent*

263   Maize yields were highly responsive to N fertilizer across crop rotation, but N  
264   response interacted with crop rotation (Table 1, Figure 5A, B). In both tillage  
265   systems, grain maize yield differences across rotations were the greatest at low to  
266   mid-N rates, and increasing the N rate to 192 kg N ha<sup>-1</sup> resulted in similar yields  
267   across all crop rotations. (Figure 5A,B). Including wheat in the MS rotation  
268   increased maize yields at the lowest N rate, while maize yields were similar at the  
269   two highest rates in both tillage systems (MS vs MSW, Figure 5A,B). Similarly,  
270   maize yields were higher in the MSW rotation than MM only at the two lowest rates  
271   in both tillage systems (Figure 5 A,B). No statistically significant maize yield gains  
272   were obtained from winter wheat with red clover (MSWrc vs MS) at N rates above  
273   or equal to 72 kg total N ha<sup>-1</sup> in zone-till (Figure 5B).

274   Increasing N rates did not alter rotation responses to tillage (Table 1); however,  
275   tillage practice altered the magnitude of the crop rotation effects obtained at  
276   various N rates (Figure 5 A,B). Except for yield benefits obtained from the inclusion  
277   of red clover into rotation, higher or similar crop rotation benefits were found at  
278   lower maximum N rates in zone-till compared to tilled systems (Table 2). Crop  
279   rotation benefits attributed to soybean (MS vs MM) were only significant in zone-  
280   tilled systems (Figure 5, Table 2) and were maximized at 90 kg N ha<sup>-1</sup> (+2416 kg  
281   ha<sup>-1</sup>, Table 2). Similarly, maximum crop rotation benefits obtained from the  
282   inclusion of soybean and wheat (MSW vs MM) were 2.1-fold higher (+1780 kg ha<sup>-1</sup>  
283   <sup>1</sup>) at N<sub>max,r</sub> rates which were 56% lower in zone-till compared to tilled systems  
284   (Table 2).

285 The highest yield gains (+2037 kg ha<sup>-1</sup>) at the lowest N<sub>max<sub>r</sub></sub> (40 kg N ha<sup>-1</sup>) were  
286 found when wheat was included into rotations of the tilled systems. The effect of  
287 wheat on the crop was further enhanced when red clover was underseeded into  
288 the wheat (Table 2). However, benefits from wheat, with or without red clover,  
289 decreased sharply with increasing N rates in zone-till systems (Figure 5B).

290

### 291 *3.3 Wheat decreases maize N requirement and improves NUE*

292 The rate of N that maximized grain maize yields (N<sub>max<sub>y</sub></sub>) and economic returns of  
293 N fertilization (MERN) tended to decrease with wheat in the crop rotations,  
294 especially in the zone-till system (Table 3). Crop rotations including wheat and red  
295 clover also reached N<sub>max<sub>y</sub></sub> and MERN values below 192 kg N ha<sup>-1</sup> in both tillage  
296 systems (Table 3). Such reductions of maize N requirements are likely  
297 underestimates because regression models were not extrapolated beyond 192 kg  
298 N ha<sup>-1</sup>, and maximum yields were often estimated at higher N rates (Figure 5A, B).  
299 However, grain yields at optimum N rates (Y<sub>N<sub>max</sub></sub>) were maintained, resulting in  
300 17% (till) to 21% (zone-till) increase in PFP<sub>MERN</sub> associated with inclusion of wheat  
301 into rotations (Table 3, MSW vs. MS). The inclusion of wheat underseeded with  
302 red clover into tilled MS rotations significantly lowered N rates required to maximize  
303 maize yields and economic returns from N fertilizer (*P*<0.05) and significantly  
304 increased PFP<sub>MERN</sub> (Table 3). Estimated grain maize yields obtained at zero-N  
305 (intercept, Y<sub>N=0</sub>) are function of the plant available soil N from net mineralization.  
306 Grain yields were significantly higher when wheat, with or without red clover, was  
307 included into rotations in both tillage systems (Table 3). The AE<sub>MERN</sub> included

308 differential plant available soil N into NUE calculations and corrected for the bias  
309 caused by the linear inverse correlation between MERN and  $Y_{N=0}$  ( $R^2=0.81$ ,  
310  $P=0.032$ ). The  $AE_{MERN}$  shows that the effects of wheat alone were likely attributed  
311 to an increase in plant available soil N, rather than an increase in efficiency of N  
312 fertilizer in both tillage systems. However, by providing a niche for red clover into  
313 the crop rotation, wheat significantly increased yield obtained per unit of N applied  
314 at MERN by 14 kg, on average, in tilled systems ( $AE_{MERN}$  MSW vs MSWrc, Table  
315 3). Incremental N fertilization had the largest impact on maize yields in rotations  
316 with wheat and red clover in the tilled system, especially at low N rates ( $AE_i$ , Figure  
317 5C). Maize yields were less responsive to N fertilization when grown in rotation  
318 with wheat in the zone-till system (Figure 5C). The  $AE_i$  showed that wheat  
319 decreases fertilizer N requirements in maize:  $AE_i$  decreased sharply with  
320 increasing N rates when wheat was in rotation, while yields of simple rotations  
321 were constrained by the maximum N rate applied, especially in tilled systems  
322 (Figure 5C, table 3). Wheat yield responses to increasing N rate were not different  
323 across rotation and tillage treatments (Table 1, Figure 6). Soybean yields were  
324 not responsive to the long-term N regimes applied in previous years to maize  
325 and/or wheat in the rotation (Table 1).

326

#### 327 **4. Discussion**

328 The objective of this study was to quantify the long-term N benefits of maintaining  
329 wheat in maize and soybean rotations under different tillage practices. Seven  
330 maize and soybean-based rotations were grown under two tillage systems and

331 four long-term N regimes in Ridgetown (ON, Canada). We show 4 years of crop  
332 performance data (2010-2013) in systems established in 1995 and at, or close-to,  
333 steady state across tillage, crop rotation, and N treatments. Steady state conditions  
334 of soil organic matter are achieved in 15-20 years of continuous practice (West  
335 and Post, 2002; Alvarez, 2005) and a meta-analysis of various long-term sites in  
336 Ontario has shown that treatments established for less than 10 years provide  
337 valuable insight into the long-term impact of crop production practices on soil  
338 properties (Congreves et al., 2014). Our results demonstrate that the value of  
339 wheat in a crop rotation with maize and soybean is much more than its market  
340 price: maintaining rotation diversity in the northern Corn Belt is instrumental to  
341 increase soybean productivity and maize yields using less N input. We found that  
342 wheat: 1) produced higher maize and soybean yields in both tillage systems  
343 (Figure 3, 4); 2) acted synergistically with conservation tillage practices to reduce  
344 the crop yield lag due to long-term zone-tillage (Figure 3, 4) and 3) decreased  
345 fertilizer N requirements for maximum maize yield (Figure 5, Table 3).

#### 346 *4.1. Wheat contribution to maize N use efficiency*

##### 347 *4.1.1 Increase in plant available soil N*

348 Wheat decreased optimal N fertilizer rates and  $PFP_{MERN}$  both directly and by  
349 providing a temporal niche for underseeding red clover (Table 3).

350 In our study, wheat N benefits could be attributed to a higher plant available soil N  
351 rather than an increase in efficiency of N fertilizer used (Table 3). Mineralization of  
352 wheat root biomass and stubble likely provided the N credit from 16 kg ha<sup>-1</sup> in the  
353 tilled system to 30 kg ha<sup>-1</sup> in the zone-till system based on MERN (MS vs MSW,

354 Table 3). Including red clover likely increased plant available soil N further (12 kg  
355 ha<sup>-1</sup> in zone-till, 83 kg ha<sup>-1</sup> in tilled system), and decreased maize N requirements  
356 (Table 3). We found similar results using a compilation of maize N responses over  
357 a range of soil type and F/P ratios for the past 40 years in Ontario (Gaudin et al.,  
358 2013). It was estimated that MERN decreased by 41–64 kg N ha<sup>-1</sup> when maize  
359 was preceded by red clover, with up to 7.11% positive rotational benefits of red  
360 clover on maize yields in conventional tillage systems (Gaudin et al., 2013).

361 Large differences in N credit obtained across the tillage treatments (Table 3) may  
362 be due to differential rates and timings of N release from above and below-ground  
363 residues (Dou et al., 1995; Groffman et al., 1987; Sarrantonio and Scott, 1988).

364 Soil nitrate levels have been reported to be higher in conventional tillage compared  
365 to zone-till throughout the growing season and incorporation of clover residue may  
366 lead to higher N release in tilled compared to zone-till systems (Dou et al., 1995;  
367 Drinkwater et al., 1998; Varco et al., 1989; Wilson and Hargrove, 1986). Moreover,  
368 other studies have shown red clover to enhance wheat straw decomposition in  
369 zone-till systems, which might have alleviated some of the negative effects of  
370 wheat residues and zone-tillage on maize emergence and yield (Drury et al., 2003,  
371 1999; Kravchenko and Thelen, 2009). However, to extract the benefits of  
372 wheat/red clover on system NUE, the release of N from above and below-ground  
373 residues must be synchronous with maize N uptake.

#### 374 *4.1.2 Increase in N fertilizer use efficiency*

375 Along with higher indigenous plant available soil N, red clover increased the  
376 efficiency of maize N fertilizer use ( $AE_{MERN} = + 14 \text{ kg grain. kg N}^{-1} \text{ applied}$ , Table 3).

377 This may be partially explained by maize preferably recovering N from fertilizer  
378 instead of legume residue decomposition, as shown in several studies evaluating  
379 N transfer from red clover to maize using N<sup>15</sup> isotopes (Gardner and Drinkwater,  
380 2009; Harris et al., 1994).

381 Direct and indirect benefits of wheat on soil properties at this trial (Van Eerd et al.,  
382 2014) may also improve recovery of N fertilizer. More diverse rotations and  
383 improvement of soil structure, aggregation and health help foster root growth  
384 (Goldstein, 2000; Nickel et al., 1995; Tisdall and Oades, 1979), which in turn may  
385 improve fertilizer N uptake (Durieux et al., 1994; Eghball and Maranville, 1993).  
386 Longer periods without tillage and abundant living plant roots in diverse rotations  
387 can also host mycorrhizae over a greater duration of time within the crop rotation  
388 (Brito et al., 2012; Curaqueo et al., 2010; Deguchi et al., 2007; Lehman et al., 2012;  
389 Kabir, 2005). This may enhance the services they provide such as increase N  
390 uptake (George et al., 1995), especially in water-stress environments (Tobar et al.,  
391 1994).

392 As a result of both increase in indigenous soil N and higher use of N fertilizer,  
393 maize yields were less limited by N supply and less responsive to N fertilization  
394 when grown in rotation with wheat, especially in the zone-till system (Figure 5B).  
395 Restrictions of N application due to regulation or weather constraints would have  
396 a smaller impact on maize yields when grown in zone-till rotations with wheat  
397 compared to MS or MM rotations. In the event of higher N fertilizer prices relative  
398 to grain maize (i.e., higher F/P ratio), NMax<sub>y</sub> would also be significantly reduced  
399 but MEY would likely remain comparable in crop rotations with wheat. Wheat may

400 therefore help mitigate the effects of lower N supply and high market volatility of N  
401 fertilizer and grain.

#### 402 *4.2. Positive feedback of lower N requirements*

403 We observed higher benefits of wheat and greater differences in maize yields  
404 across rotations at low to mid-N rates (Table 2). Various researchers have reported  
405 lower beneficial effect of crop rotation on maize yield with increasing N fertilization,  
406 especially when legumes were included (Adams et al., 1970; Copeland and  
407 Crookston, 1992; Crookston et al., 1991; Nevens and Reheul, 2001; Peterson and  
408 Varvel, 1989; Porter et al., 1997; Riedell et al., 1998; Singer and Cox, 1998;  
409 Stecker et al., 1995). In our study, red clover plant population densities significantly  
410 decreased with increasing wheat N rates (from 165 plants m<sup>-2</sup> at zero-N to 18  
411 plants m<sup>-2</sup> at 150 kg N ha<sup>-1</sup> across tillage systems and years, data not shown).  
412 Lower rates of N fertilizer in wheat have been shown to increase red clover  
413 biomass and stand count and decrease clover patchiness (Gaudin et al., 2014).  
414 As a result, lower N rates maximize economic returns from wheat-red clover  
415 intercropping with higher partial profits (Gaudin et al, 2014). Yet, wheat rotation  
416 benefits on maize yield were masked by high N rates (Figure 5, A-B), implying that  
417 higher N applications could offset the negative impact of monoculture or short  
418 rotation. However, we show that similar yields can be obtained with lower N levels  
419 in rotationally grown maize and that soybean yields, which were not responsive to  
420 increasing N rates, significantly benefited from any rotation with wheat (Figures 4,  
421 5). If N requirements are lower when crops are grown in rotation, the potential risk  
422 of nitrate losses through leaching or denitrification may also be reduced (Varvel

423 and Peterson, 1990; Yamoah et al., 1998b). These results highlight the value of  
424 wheat as a system component and its potential to increase both maize and  
425 soybean productivity using less N input. It also suggests that fertilizer N levels  
426 should be taken into account when comparing crop rotation benefits across studies  
427 and environments.

#### 428 *4.3. Additional value of wheat on soybean yields*

429 Our study also supports the hypothesis that crop productivity is increased with crop  
430 rotation diversity. The inclusion of wheat in a MS or SS rotations significantly  
431 increased soybean yields by an average of 0.47 Mg ha<sup>-1</sup> across tillage systems  
432 (Figure 4). Higher soybean yields may be attributed to the benefits of small grain  
433 cereal on soil structure. Soil quality parameter such as aggregate stability,  
434 penetrometer resistance and other parameters used for the Cornell Soil Health  
435 Assessment, were found higher with a higher frequency of winter wheat in the  
436 rotation at this trial (Van Eerd et al., 2014). Lowest soybean yields were produced  
437 with continuous soybean in the tilled system (Figure 4), which corresponded with  
438 the treatment with the lowest soil quality (Van Eerd et al., 2014). Others have  
439 reported higher soybean yields in systems that retain soil moisture (Pedersen and  
440 Lauer, 2004), which may have occurred with wheat in this study because of  
441 improved soil structure. Wheat might also help break pest cycles and decrease  
442 the incidence of soil-borne pathogens and soybean cyst nematodes, which can  
443 negatively impact on soybean yields (Zhang et al., 2012).

#### 444 *4.4. Conclusions and significance for northern Corn Belt cropping systems*

445 Crop diversity regulates various bioprocesses such as residue decomposition and

446 microbial dynamics with large effects on nutrient cycling (McDaniel et al., 2014).  
447 Given the large production areas of maize and soybean in the northern Corn Belt  
448 (Figure 1), diversifying continuous or short maize and soybean rotations with wheat  
449 has potential to increase NUE of agricultural systems and alleviate N  
450 environmental footprint at a large scale. However, more research and economic  
451 analysis is needed to quantify opportunity costs, wheat winter survival in other  
452 states of the Corn Belt and confirm potential at the Corn Belt scale. Nonetheless,  
453 wheat provides a valuable temporal niche to include late-season legumes, such as  
454 red clover, into northern cropping systems and obtain numerous non-N benefits  
455 (Gaudin et al., 2013). For instance, it has been shown recently that forages, wheat  
456 and other small grain cereals help mitigate weather variations and improve maize  
457 and soybean yield stability in Ontario, especially when hot and dry conditions occur  
458 (Gaudin et al, 2015). This is highly significant to maintain yields as springs may  
459 become wetter, summers drier and hotter, with greater frequencies of abnormally  
460 low precipitation or high temperature extremes (Hatfield et al., 2013; IPCC, 2013).  
461 Finally, advances in crop breeding will be realized more efficiently when higher  
462 crop yields are produced in diverse crop rotations, especially in the general context  
463 of decreased N inputs and higher environmental stresses.

464

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474

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## 715 **7. Figure legend**

716 **Figure 1. Harvested areas of field crops grown in four states/provinces of the**  
717 **northern Corn Belt from 1981-2013.** Harvested areas (hectares) of major field  
718 crops are shown as % of total harvested area from 1981- 2013 for Ontario (  
719 OMAFRA, 2014), Michigan, Minnesota and Iowa (USDA-NASS, 2014b). Surface  
720 areas harvested in canola and hay were not included for clarity.

721 **Figure 2. Weather conditions at the experimental site.** (A) Monthly 10-year

722 average temperature (bars) and precipitation (line) pattern (2003-2013). Mean  
723 temperature (A) and total precipitation (B) deviation from 10-year average during  
724 crop growth (March to November) for each study year (2010-2013). Weather data  
725 was collected daily by Environment Canada at the site.

726 **Figure 3. Yearly variation in maize yields response to rotation and tillage.** LS  
727 means  $\pm$  SE across N treatment are shown. Letters indicate statistical differences  
728 among rotations within tillage treatment for each year and (\*) indicate significant  
729 tillage effect for each rotation at  $p=0.05$ . Crop abbreviation: S= soybean, M=maize,  
730 W=winter wheat, Wrc = winter wheat underseeded with red clover.

731 **Figure 4. Soybean yields response to rotation and tillage.** LS means (2010-  
732 2013)  $\pm$  SE across N treatment are shown. Letters indicate statistical differences  
733 among rotations within tillage treatment and (\*) indicate significant tillage effect for  
734 each rotation at  $p=0.05$ . Crop abbreviation: S= soybean, M=maize, W=winter  
735 wheat, Wrc = winter wheat underseeded with red clover.

736 **Figure 5. Impact of crop rotation and tillage on maize yields response to N**  
737 **fertilization.** Regression analysis of the effects of N rate treatments on maize  
738 yields under (A) conventional tillage and (B) zone tillage systems. Maize yield  
739 response to N rates (2010-2013) was fitted using quadratic plateau models capped  
740 at  $192 \text{ kg N ha}^{-1}$ . Nitrogen rates maximizing yields for each rotation and tillage  
741 treatments are presented in Table 3. Markers show treatment LS means used for  
742 regression analysis  $\pm$  SE. (C) Agronomic efficiency of N fertilization ( $AE_i$ ).  
743 Agronomic efficiencies are estimated yield gain per unit of increasing N fertilization  
744 based on N response equations (A-B). Crop abbreviation: S= soybean, M=maize,

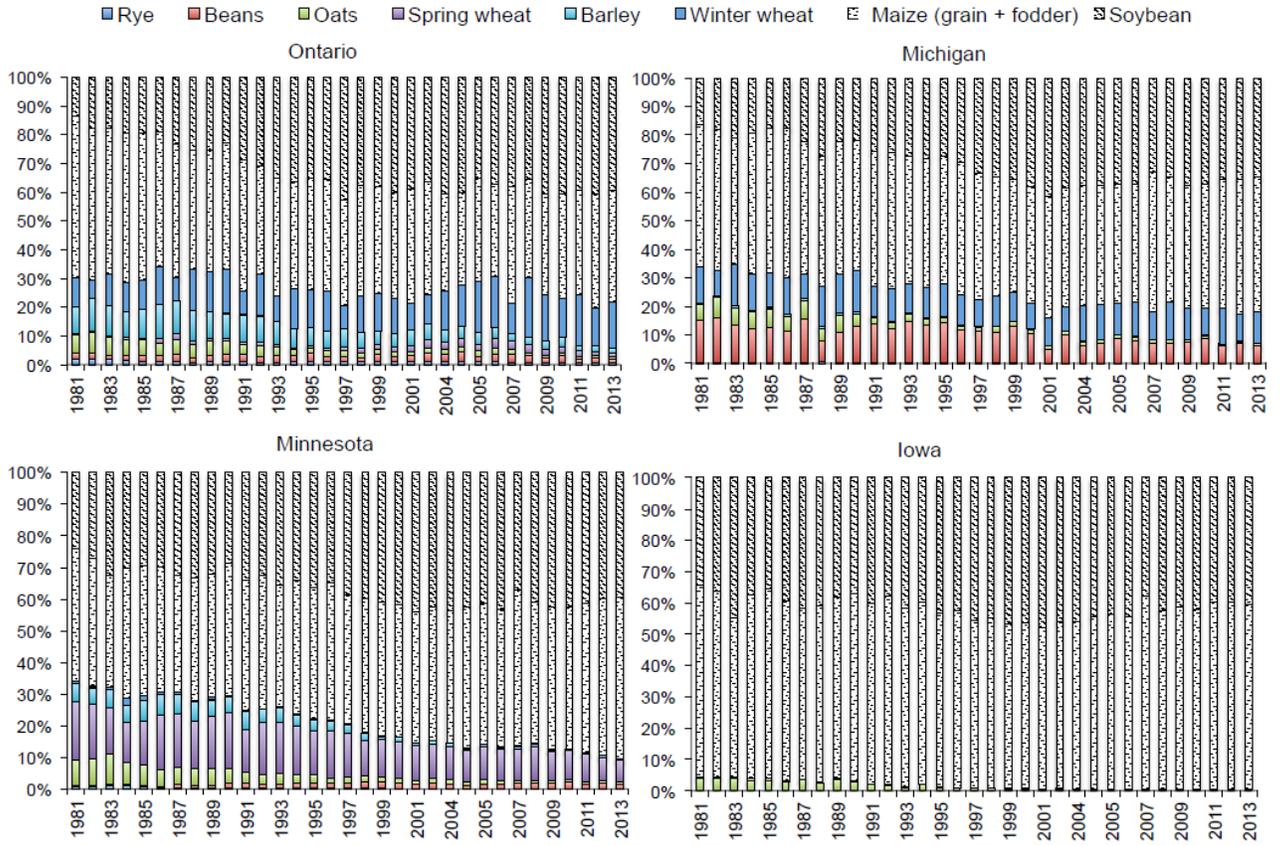
745 W=winter wheat, Wrc = winter wheat underseeded with red clover.

746 **Figure 6. Impact of crop rotation and tillage on wheat yields response to N**  
747 **fertilization.** (A-B) Regression analysis of the effects of N rate treatments on  
748 wheat yields (2010-2013) under (A) conventional tillage and (B) zone tillage  
749 systems. Wheat yield response to N rates was fitted using quadratic plateau  
750 models capped at 150 kg N ha<sup>-1</sup>. Markers show treatment LS means used for  
751 regression analysis ± SE. Crop abbreviation: S= soybean, M=maize, W=winter  
752 wheat, Wrc= winter wheat underseeded with red clover.

753 **Figure A.1. Changes in crop-specific rotation benefits with N fertilization.** (A-  
754 B) Regression analysis of the effects of N rate treatments on maize yield benefits  
755 associated with different crops under (A) conventional tillage and (B) zone tillage  
756 systems. Maize yield response to N rates was fitted using quadratic plateau  
757 models capped at 0 and 192 kg N ha<sup>-1</sup>. Regression models and N rates maximizing  
758 yield benefits for each crop are presented in Table 2. Crop abbreviation: S=  
759 soybean, M=maize, W=winter wheat, Wrc= winter wheat underseeded with red  
760 clover.

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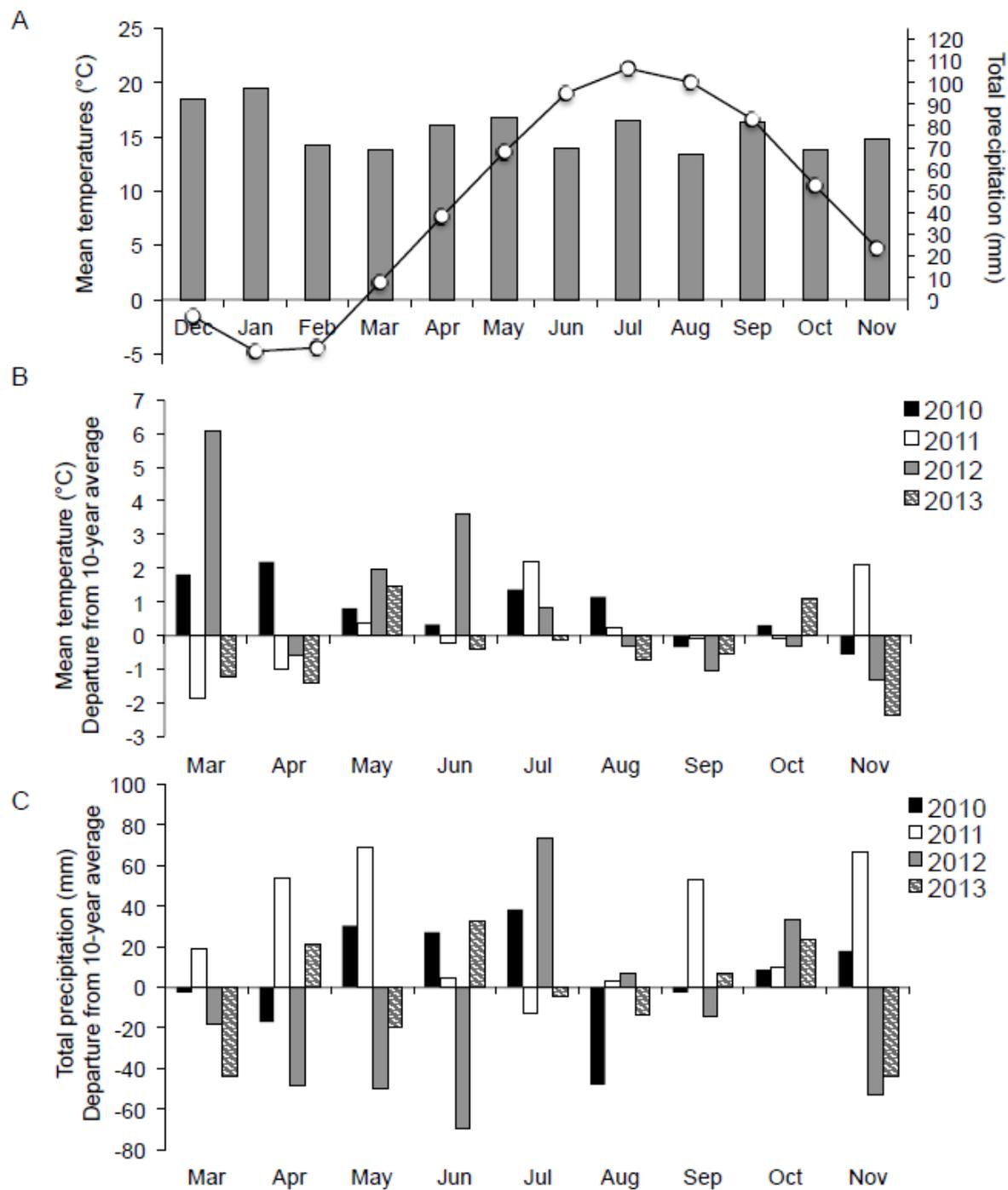
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764 **Fig 1**

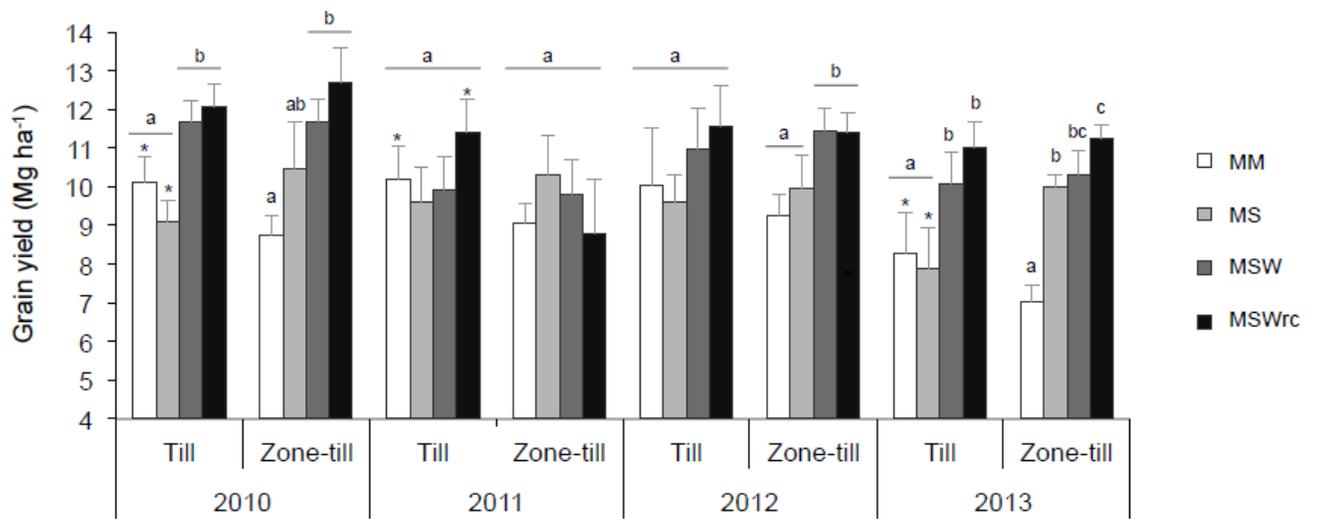
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767 **Fig 2**

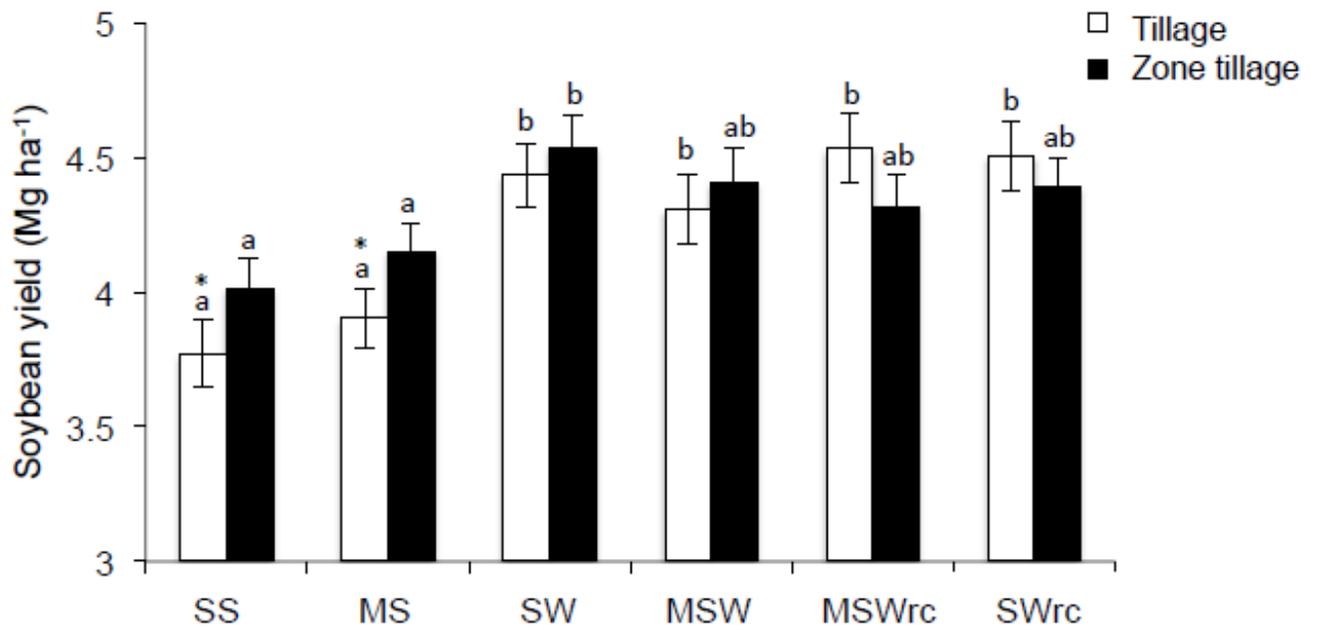
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770 **Fig 3**

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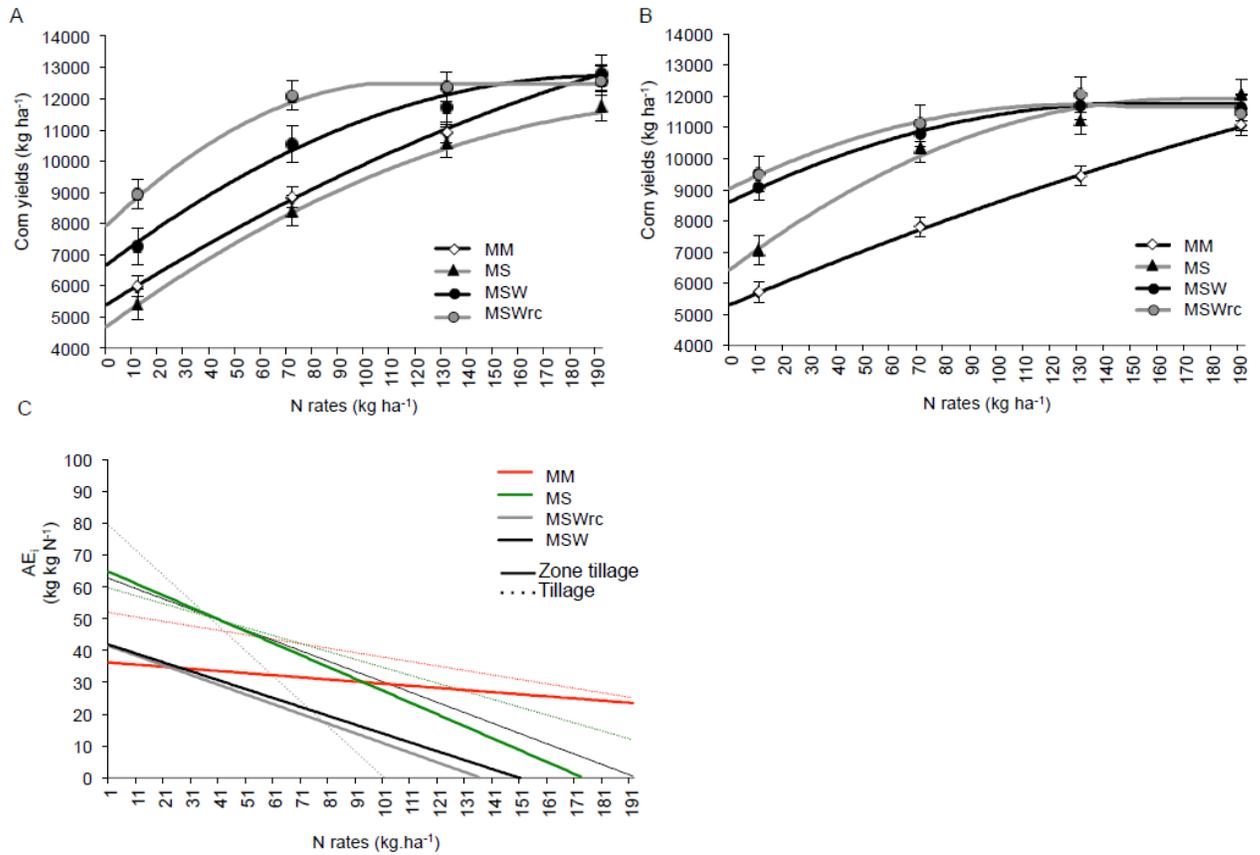


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773 **Fig 4**

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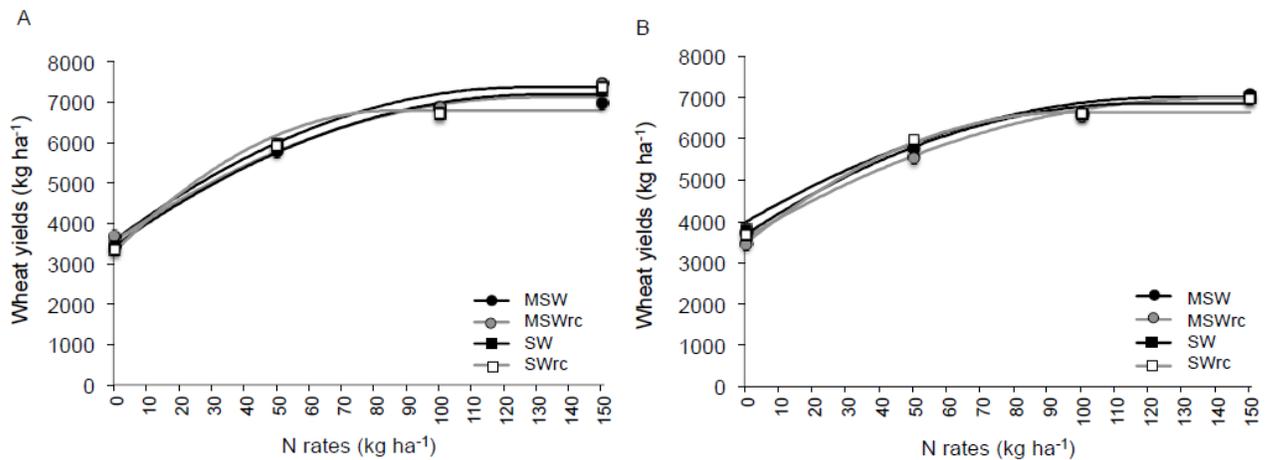
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777 **Fig 5**

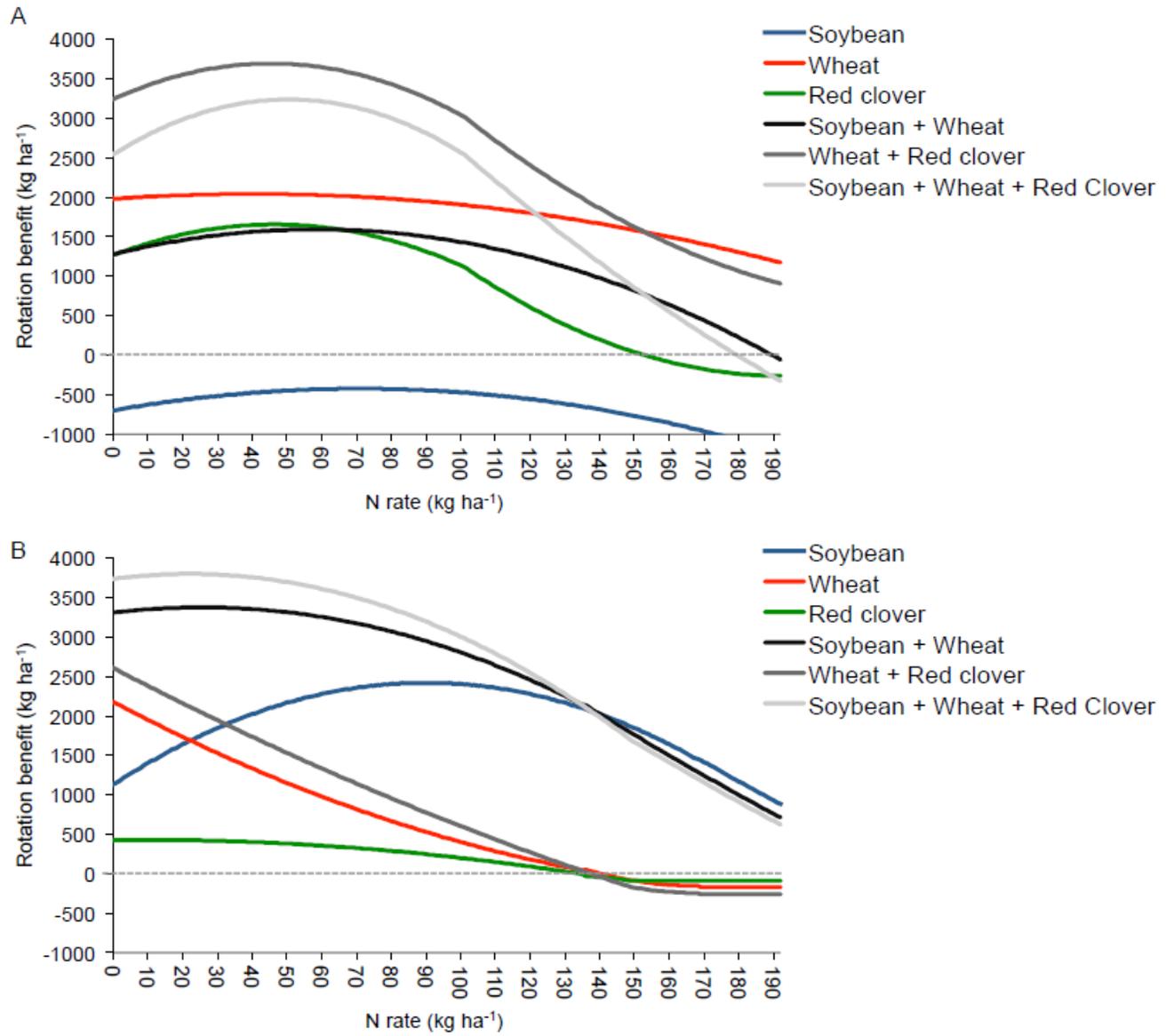
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780 **Fig 6**

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783 **Fig A1**

784 **Table 1.** Long term rotation, tillage and nitrogen effects on maize, soybean and wheat yields grown from 2010-2013.

	Maize yield		Soybean yield		Wheat yield	
	df	Pr (>F)	df	Pr (>F)	df	Pr (>F)
Year	3	< 0.001 **	2	< 0.001 **	1	0.021 *
Tillage	1	0.801	1	0.713	1	0.286
Rotation	3	< 0.001 **	5	< 0.001 **	3	0.090
Nitrogen <sup>□a</sup>	3	< 0.001 **	3	0.477	3	< 0.001 **
Year x Tillage	3	0.044 *	2	0.079	1	0.773
Year x Rotation	9	< 0.001 **	10	0.004 *	3	< 0.001 **
Year x Nitrogen	9	< 0.001 **	6	0.068	3	< 0.001 **
Tillage x Rotation	3	0.016 *	5	0.008 *	3	0.731
Rotation x Nitrogen	9	< 0.001 **	15	0.387	9	0.555
Nitrogen x Tillage	3	< 0.001 **	3	0.345	3	0.071
Year x Tillage x Rotation	9	0.105	10	0.539	3	0.184
Year x Rotation x Nitrogen	27	0.301	30	0.614	9	0.324
Year x Nitrogen x Tillage	9	0.119	6	0.499	3	0.278
Tillage x Nitrogen x Rotation	9	0.535	15	0.338	9	0.594
Maize: 0 kg N ha <sup>-1</sup>	7	< 0.001 **		N/A		N/A
72 kg N ha <sup>-1</sup>	7	< 0.001 **		N/A		N/A
132 kg N ha <sup>-1</sup>	7	< 0.001 **		N/A		N/A
192 kg N ha <sup>-1</sup>	7	0.089		N/A		N/A
Wheat: 0 kg N ha <sup>-1</sup>		N/A		N/A	7	0.830
50 kg N ha <sup>-1</sup>		N/A		N/A	7	0.112
100 kg N ha <sup>-1</sup>		N/A		N/A	7	0.582
150 kg N ha <sup>-1</sup>		N/A		N/A	7	0.124
Year x Tillage x Rotation x Nitrogen	27	0.528	30	0.522	9	0.209

785

786 (a) Nitrogen rates directly applied to maize and wheat or as part of the rotation history for soybean.

787 (\*) Significant at 0.05 probability level, (\*\*) Significant at <0.001 probability level, (N/A) Non applicable

788 **Table 2.** Effects of N fertilization on crop-specific rotational benefits to maize yields

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Rotation benefits	$\Delta$ yield	Nmax <sub>r</sub> (kg N ha <sup>-1</sup> )		Y <sub>r</sub> at Nmax <sub>r</sub> (kg ha <sup>-1</sup> )		Regression models (Capped at 0 and 192 kg N ha <sup>-1</sup> )	
		Till 	Zone Till	Till	Zone Till	Till	Zone Till
Soybean (S)	MS-MM	71	90	-426	2416	$Y_b = -702 + 7.77N - 0.0548N^2$	$Y_b = 1227 + 28.63N - 0.1591N^2$
Wheat (W)	MSW-MS	40	0	203	2176	$Y_b = 1976 + 3.04N - 0.0377N^2$	$Y_b = 2175 - 23.32N + 0.0557N^2$
Red clover (RC)	MSWrc-MSW	47	8	165	429	$Y_b = 1262 + 16.77N - 0.1804N^2$	$Y_b = 427 + 0.41N - 0.0269N^2$
S + W	MSW-MM	58	26	159	3371	$Y_b = 1274 + 10.81N - 0.0926N^2$	$Y_b = 3303 + 5.31N - 0.1033N^2$
W + RC	MSWrc-MS	45	0	368	2603	$Y_b = 3239 + 19.81N - 0.2182N^2$	$Y_b = 2603 - 22.91N + 0.0289N^2$
S + W + RC	MSWrc-MM	50	22	323	3793	$Y_b = 2537 + 27.58N - 0.2730N^2$	$Y_b = 3730 + 5.72N - 0.1302N^2$

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791 Nitrogen rates maximizing rotation effect (Nmax<sub>r</sub>) were calculated based on the regression models. Corresponding  
 792 regression curves are shown in Figure A.3. Abbreviations: Y<sub>r</sub>= Yield gain from rotation crops; S= soybean, M=maize,  
 793 W=winter wheat, Wrc = winter wheat underseeded with red clover.

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795 **Table 3.** Effect of crop rotation on maize N use and economic optimums

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Rotation	N <sub>maxY</sub> (kg N ha <sup>-1</sup> )		Y <sub>Nmax</sub> (kg ha <sup>-1</sup> )		MERN (kg N ha <sup>-1</sup> )		MEY (kg ha <sup>-1</sup> )		PFP <sub>MERN</sub> (kg grain kg N <sup>-1</sup> )		Y <sub>N=0</sub> (kg ha <sup>-1</sup> )		AE <sub>MERN</sub> (kg grain kg N <sup>-1</sup> )	
	Till	Zone Till	Till	Zone Till	Till	Zone Till	Till	Zone Till	Till	Zone Till	Till	Zone Till	Till	Zone Till
MM	192 <sup>a</sup>	192	12730 <sup>*</sup>	11039	192 <sup>a</sup>	192 <sup>a</sup>	12730 <sup>*</sup>	11039	66	57	5386 <sup>a</sup>	5299 <sup>a</sup>	38	30
MS	192 <sup>a</sup>	174	11786	11920	192 <sup>a</sup>	154 <sup>a</sup>	11786	11853	61	77	4684 <sup>a</sup>	6427 <sup>b</sup>	37	35
MSW	192 <sup>a</sup>	150	12710	11660	176 <sup>ab</sup>	124 <sup>ab</sup>	12638	11570	72	93	6661 <sup>b</sup>	8603 <sup>c</sup>	34	24
MSWrc	101 <sup>b</sup>	136	12465	11749	93 <sup>b</sup>	112 <sup>b</sup>	12437	11666	133	104	7924 <sup>c</sup>	9030 <sup>c</sup>	48	23

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798 The effects of crop rotation on maize nitrogen use and economic optimums were calculated based on the regression models  
 799 shown in Figure 3 and not extrapolated beyond 192 kg N ha<sup>-1</sup>. Estimates within each variable followed by similar letter or  
 800 no letters were not significantly different at p=0.05. (\*) Indicates significant tillage effect at p = 0.05. Abbreviations: N<sub>maxY</sub>  
 801 = Nitrogen rates at maximum grain yield; Y<sub>Nmax</sub>= Grain yields at N<sub>maxY</sub>; MERN = Maximum Economic Rate of Nitrogen;  
 802 MEY = Maximum Economic Yield (estimated grain yield at MERN), PFP<sub>MERN</sub> = Partial Factor Productivity for N fertilizer at  
 803 MERN (MEY/MERN); Y<sub>N=0</sub> = Yield at N=0, AE<sub>MERN</sub> = Agronomic Efficiency of N fertilizer at MERN ((MEY- Y<sub>N=0</sub>)/MERN); M  
 804 = Maize; S= Soybean; W=Winter Wheat; Wrc= Winter Wheat underseeded with red clover.

805 **Table A.1.** Planting and harvest dates of cultivars from 2009 to 2013.

	Crop year	Planting date	Harvest date	Cultivar
Maize	2009	25 May	24 Nov	Pioneer 35F40
	2010	27 May	12 Nov	Pioneer 37V63
	2011	8 June	14 Nov	Pioneer P0474HR
	2012	26 April	14 Nov	Pioneer P0891XR
	2013	14 May	7 Nov	Pioneer P0891XR
Soybean	2009	25 May	13 Oct	NK S21-N6
	2010	31 May	7 Oct	Dekalb DKC30-07
	2011	8 June	7 Oct	Pioneer 92Y30
	2012	23 May	28 Sept	Pioneer 92Y30
	2013	22 May	25 Sept	Pioneer 91Y81
Winter Wheat	2009	15 Oct	29 July	Pioneer 25R47
	2010	14 Oct	15 July	Pioneer 25R47
	2011	19 Oct	14 July	Pioneer 25R56
	2012	2 Nov	10 July	Pioneer 25R40
	2013	3 Oct	16 July	Pioneer 25R40

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