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Article

Investigating the Combined Effect of Tillage, Nitrogen Fertilization and Cover Crops on Nitrogen Use Efficiency in Winter Wheat

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Abstract: A field study was conducted in northern France over two consecutive years to evaluate the combined effect of conventional tillage (CT) vs no till (NT) with or without cover crops (cc) and nitrogen (N) fertilization on various agronomic traits related to N use efficiency in winter wheat. Five years after conversion of CT to NT, significant increases in N use efficiency, N utilization efficiency, N agronomic efficiency, N partial factor productivity, N apparent recovery fraction and N remobilization were observed under three N fertilization regimes (0, 161, 215 kg ha^{-1}). It was also observed that grain yield and grain N content were similar under CT and NT. The N nutrition index was higher under NT at the three rates of N fertilization. Moreover, N use efficiency related traits were increased in the presence of cc both under NT and CT. Thus, agronomic practices based on continuous NT in the presence of cc, appear to be promising strategies to increase N use efficiency in wheat, while reducing both the use and the loss of N-based fertilizers.

Keywords: nitrogen use efficiency; tillage system; cover crops; nitrogen application; grain yield; winter wheat

1. Introduction

Over the last 50 years, nitrogen (N) fertilizers have been used extensively to increase wheat yield [1]. The imbalance between the amount of N applied and the ability of the crop to utilize added N fertilizers can lead to residual soil N accumulation [2–4]. It has been estimated that only half of the applied N inputs are recovered in harvested crops and their residues [5]. The N remaining in the soil can be lost by runoff, erosion, leaching or gaseous loss through denitrification and volatilization [6], which can have a detrimental impact on the environment [7,8].

Various parameters are commonly used in agronomic research to evaluate the efficiency of the response of crops to applied N. Both from a physiological and agronomic point of view, N use efficiency (NUE) is the result of two main biological processes: N uptake efficiency (NUpE), which is the ability of a crop to produce yield per unit of N available in the soil, and N utilization efficiency (NUtE) which is the ability of a crop to produce yield per unit of N taken up [9–14]. In field studies, other parameters can be calculated based on differences in crop yield or total N uptake between fertilized plots and unfertilized controls using the 'difference method' [12,15–19]. Among them, agronomic efficiency of N (AEN), which corresponds to the increase in biomass or yield per unit of N applied and partial factor productivity of N (PFPN), which corresponds to the capacity of a plant to produce biomass or yield per hectare divided by the rate N applied, have been used to monitor the performance of agricultural management practices. In addition, the N apparent recovery fraction (NAR) corresponds to the increase in plant N uptake per unit of N applied, which depends on the congruence between plant N demand and the quantity of N released from the applied N fertilizer. Because each of the parameters representative of NUE has a different meaning from an agronomic or physiological point of view, it is generally recommended to use all of them in order to identify possible variations in NUE [15].

The N nutritional status of a crop, in particular N deficiency, can be monitored using the N nutrition index (NNI) [20], that can be calculated using species-specific N dilution curves [21]. Monitoring the crop N nutritional status using NNI [22] allows the application of N fertilizers to coincide with the N requirement of the crop [23], which can reduce N losses to the environment [24].

In the last three decades, improving NUE has been one of the most important challenges in modern agriculture. Therefore, various technological and agronomic approaches have been developed in parallel with improving our understanding of the genetic and physiological basis of NUE for further breeding and agronomic applications [25]. Among the agronomic approaches, it has been emphasized that short- and long-term agricultural management practices must be further developed for sustainable crop production, using N more efficiently. A number of these agricultural practices are based on the development of soil conservation, a technique known to improve the uptake of soil N by the plant [10,26] and to increase the content of soil organic matter (SOM) [27–32].

In the present study, the combined effects of tillage with or without cover crops cc and N inputs on wheat NUE related traits and N nutrition were evaluated. The changes observed in NNI and NUE traits, such as NUtE, AEN, PFPN and NAR allowed the development of a strategy for maintaining wheat productivity in a sustainable agricultural system, based on the rationalization of N fertilizer usage.

2. Results

2.1. Effect of Tillage Treatment and N Fertilizer Rate on Agronomic Traits

To evaluate the impact of tillage and N fertilization on wheat productivity, agronomic data including aboveground biomass, grain yield and grain N content were first subjected to variance analysis. Two-way ANOVA with tillage practice with or without cc and N fertilization as the two main parameters. Similar results were obtained when performing three-ways ANOVA using cc as a third parameter was carried out using the two soil management practices, continuous till (CT), with or without cc and no-till (NT), with or without cc as the first parameter and the level of N application (N0, N1, N2) as the second parameter.

Over the two years of experimentation, N application significantly increased aboveground biomass production, grain yield and grain N content (Table 1). For the three agronomic traits, the increase was on average around 3-fold for N1 and 4-fold for N2 compared to the non-fertilized plots (N0). For example, above ground biomass production in the different NT and CT treatments with or without cc was on average 4617 kg ha^{-1} in N0, 15,847 kg ha^{-1} in N1 and 17,940 kg ha^{-1} in N2.

Over the two years of experimentation, when the CT treatment was compared to the NT treatment, only the aboveground plant biomass was significantly increased in the N0 treated plants grown with or without cc. Grain yield was not modified in 2014 irrespective of the tillage conditions, whilst in 2015 an increase in grain yield was observed in N1 under NT with or without cc. The tillage condition did not have any impact on grain N content.

Overall, the presence of cc did not modify plant productivity and grain quality except in 2015, when cc caused an increase in aboveground plant biomass and grain yield only under CT conditions when the plant received the highest amount of fertilizers (N2). On the same year, such an effect was also observed in N1 under NT conditions. In 2014, under N2 fertilization conditions, an increase in the grain N content was only observed under CT in the presence of cc.

Table 1. Impact of tillage, cover crops and N fertilization on agronomic traits.

Source of Variance		**2014**			**2015**		
N Fertilizer Rate	**Tillage Practice**	**AG Biomass (kg ha^{-1})**	**Grain Yield (kg ha^{-1})**	**Grain N (kg ha^{-1})**	**AG Biomass (kg ha^{-1})**	**Grain Yield (kg ha^{-1})**	**Grain N (kg ha^{-1})**
N0	NT	3520 f	1869 c	22.76 e	4541 g	2298 g	26.47 e
	NTcc	3499 f	2467 c	34.38 e	5229 g	2492 fg	28.69 e
	CT	5868 e	2228 c	34.92 e	5870 fg	2548 fg	28.88 e
	CTcc	5583 e	2530 c	37.05 e	7192 f	3495 f	38.97 e
N1	NT	13,921 d	6463 a	93.48 dc	15,637 e	8907 d	118.3 cd
	NTcc	15,434 cd	8109 ab	118.9 bc	19,084 cd	10,123 bc	129.2 bc
	CT	17,805 ab	8390 ab	115.7 c	16,039 e	7627 e	105.5 d
	CTcc	16,231 bc	8534 ab	119.6 bc	15,891 e	7645 e	103.5 dc
N2	NT	18,194 ab	8967 ab	118.8 bc	21,588 ab	11,560 a	171.6 ab
	NTcc	17,196 abc	9065 ab	137.4 ab	22,440 a	11,816 a	177.5 a
	CT	17,409 abc	8409 ab	114.2 c	18,206 d	9175 cd	141.2 b
	CTcc	18,936 a	10,069 a	143.8 a	20,453 bc	11,061 ab	170.4 a
Analysis of variance				$p > F$			
Tillage practice		<0.01 **	ns	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
N fertilizer		<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Tillage practice × N fertilizer		ns	ns	ns	<0.001 ***	<0.001 ***	<0.001 ***

NT: No till without cover crops. NTcc: No till with cover crops. CT: Conventional tillage without cover crops. CTcc: Conventional tillage with cover crops. N0: no added N fertilization. N1: 161 kg N ha^{-1}. N2: 215 kg N ha^{-1}. AG: aboveground. Soil tillage practice (CT and NT) and N fertilization (N0, N1 and N2) were subjected to variance analysis (Two-way ANOVA, with tillage practice with or without cc and N fertilization as the two main parameters). Treatment means were compared using a Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter within a column are not significantly different. (**, *** = significant at 0.01, 0.001 probability level, respectively), ns = not significant.

2.2. Effect of Tillage Treatment and N Fertilizer Rate on Different NUE Indices

Both in 2014 and 2015, an increase in NUE (20% on average), was observed in the absence of N fertilization (N0), irrespective of the tillage practice and of the presence of cover crops (Figure 1). Over the two years of experimentation, CT had a significant negative impact on NUE following N0 and N2 fertilization compared to NT. Under N1 fertilization, such a negative impact was only observed in 2015. Cover crops had a positive impact on NUE over the two years of experimentation under NT and N1 fertilization conditions. Such a positive impact of cover crops was observed in 2014 following N2 fertilization and in 2015 following N0 fertilization irrespective of the tillage practices (Figure 1).

In 2015, an increase in NUtE was observed when the levels of N fertilization were decreased irrespective of the tillage practice and of the presence of cc, except for NT in N1 which was higher than CT in N0. Tillage had a negative impact on NUtE in 2014 and in 2015, but only in the absence of N fertilization (N0). In N1, such a negative impact on NUtE was only observed in 2015. Cover crops had a positive effect on NUtE in 2015, irrespective of the tillage conditions in the absence of N fertilization. In 2015, such positive effect on NUtE was also observed under CT with cc only following N2 fertilization and under NT in N1with or without cc (Figure 2).

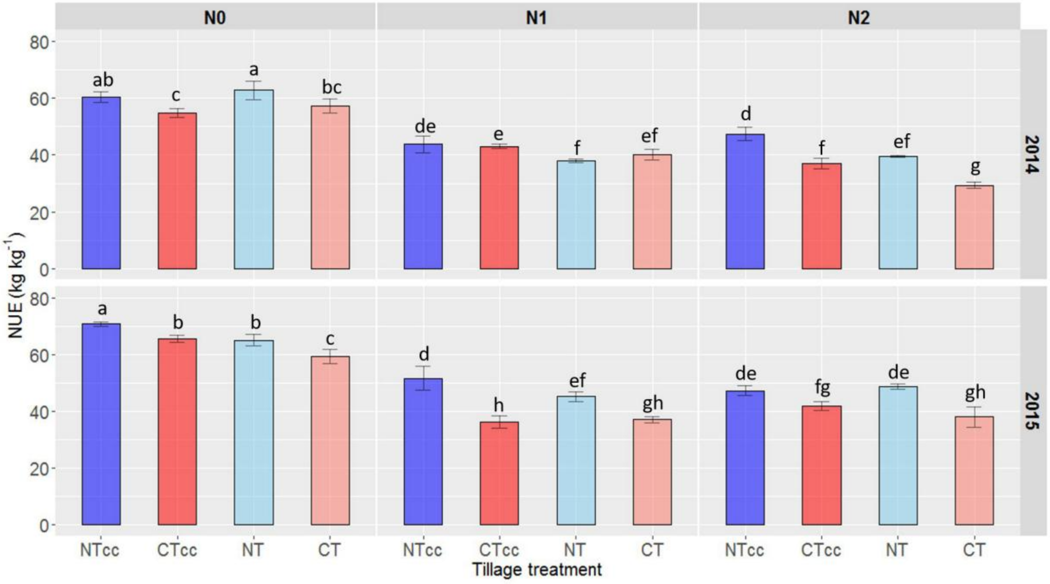

Figure 1. Effect of soil tillage treatment, cover crops and N application on NUE of wheat grown in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no added N fertilization. N1: 161 kg N ha^{-1}. N2: 215 kg N ha^{-1}. Soil management practices (CT and NT) and N fertilization (N0, N1 and N2) were subjected to analysis of variance (two-way ANOVA, with tillage practice with or without cc and N fertilization as the two main parameters). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different across N fertilizer treatments.

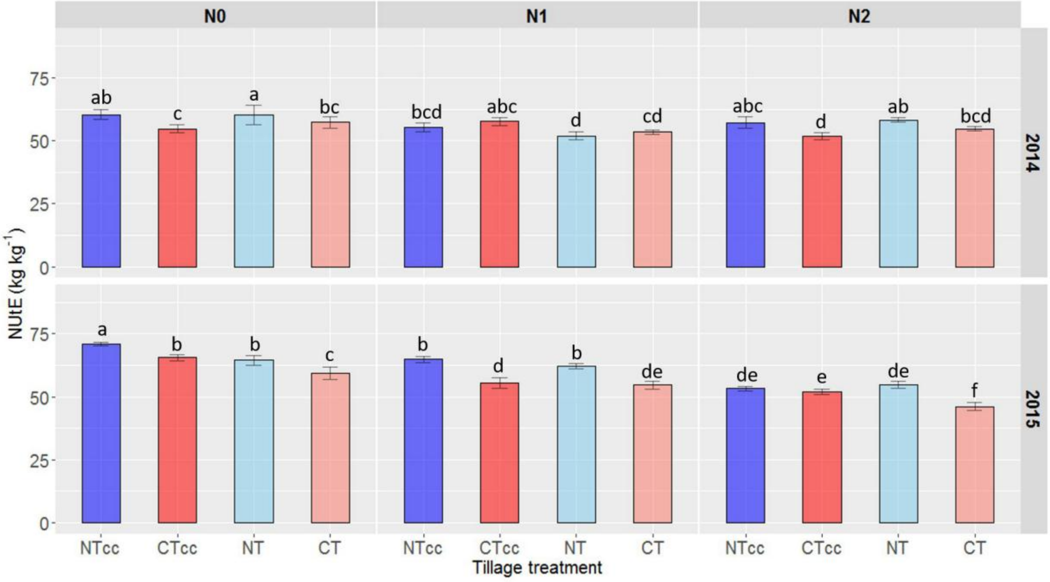

Figure 2. Impact of soil tillage treatment, cover crops and N application on NUtE of wheat grown in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no added N fertilization. N1: 161 kg N ha^{-1}. N2: 215 kg N ha^{-1}. Soil tillage practices (CT and NT) and N fertilization (N0, N1 and N2) were subjected to analysis of variance (two-way ANOVA, with tillage practice with or without cc and N fertilization as the two main parameters). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different across N fertilizer treatments.

Both in 2014 and 2015, greater amounts of N (around 3-times) were remobilized (NRem) when N fertilizers were applied (N1 and N2). Over these two years, higher amounts of N (20% increase) were also remobilized under NT in the presence of cc in N1. In N2, such positive effect of NT on NRem was only observed in 2015. In the same year NRem increased around 20% in the presence of cc both under NT and CT conditions (Figure 3).

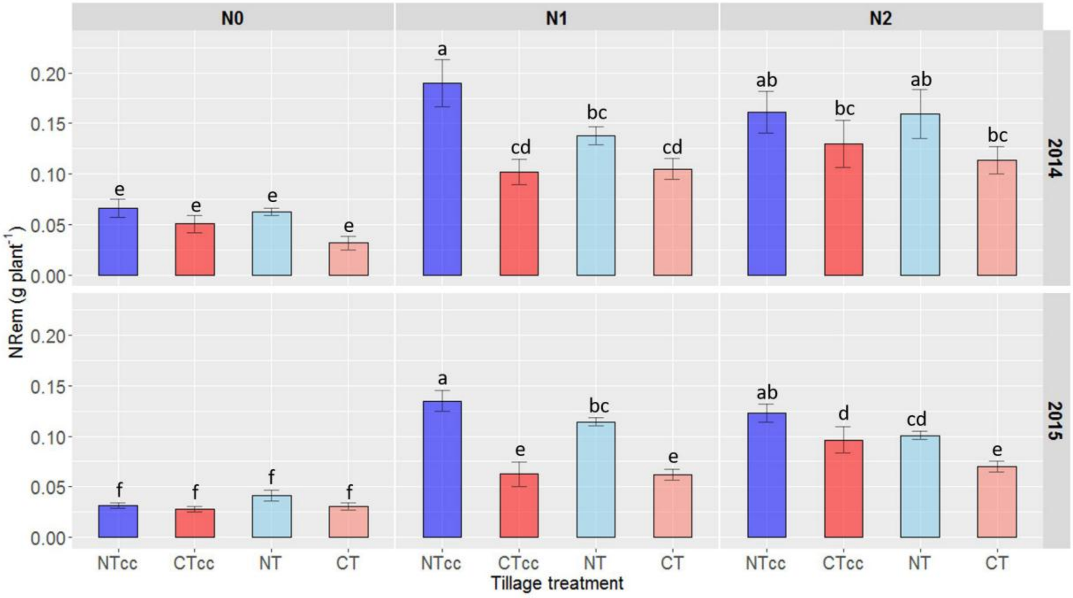

Figure 3. Impact of tillage treatment, cover crops and N application on NRem of wheat grown in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no added N fertilization. N1: 161 kg N ha^{-1}. N2: 215 kg N ha^{-1}. Soil tillage practices (CT and NT) and N fertilization (N0, N1 and N2) were subjected to analysis of variance (two-way ANOVA with tillage practice with or without cc and N fertilization as the two main parameters). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different across N fertilizers treatments.

Conventional tilling had a negative impact on AEN in N2 both in 2014 and 2015 (Table 2). In N1, CT had a negative effect on AEN only in 2015. In 2014, the presence of cc increased AEN under both CT and NT conditions, following N2 fertilization. Such a cc-dependent increase in AEN was also observed in 2015 but only following N1 fertilization (Table 2).

PFPN was significantly lower under CT both in N1 and in N2 only in 2015 irrespective of the presence of cc. A decrease in PFPN was also observed in 2014 under the two N fertilization conditions, but only in the absence of cc (Table 2).

A decrease in NAR was observed over the two years of experimentation under CT conditions only in N2. Under CT, NAR was also lower in N1 but only in 2015. The presence of cc had a limited impact on the NUE trait as NAR increased only in 2014 following N2 treatment (Table 2).

Table 2. Impact of tillage practices and nitrogen fertilization on NUE indices.

Source of Variance		2014			2015		
N Fertilizer	**Tillage Practice**	**AEN (kg kg^{-1})**	**PFPN (kg kg^{-1})**	**NAR (%)**	**AEN (kg kg^{-1})**	**PFPN (kg kg^{-1})**	**NAR (%)**
N1	NT	42.27 ab	56.01 ab	62.85 ab	42.41 c	54.37 bc	88.24 de
	NTcc	42.25 ab	59.26 a	66.06 a	55.73 a	69.54 a	89.8 cd
	CT	36.25 bc	53.10 b	60.43 abc	31.81 d	48.53 d	80.23 f
	CTcc	40.58 abc	57.19 ab	64.37 a	25.78 e	47.49 d	75.66 ef

Table 2. *Cont.*

Source of Variance		2014			2015		
N Fertilizer	**Tillage Practice**	**AEN ($kg\ kg^{-1}$)**	**PFPN ($kg\ kg^{-1}$)**	**NAR (%)**	**AEN ($kg\ kg^{-1}$)**	**PFPN ($kg\ kg^{-1}$)**	**NAR (%)**
N2	NT	36.21 b	44.37 c	56.68 bc	46.16 b	56.06 b	64.55 ac
	NTcc	50.41 a	44.14 c	61.39 ab	43.37 bc	54.96 bc	70.40 a
	CT	25.62 d	34.65 d	48.64 d	34.23 d	48.16 d	56.28 b
	CTcc	33.40 c	43.50 c	53.75 c	35.19 d	51.45 cd	60.22 bc
Analyse of variance					$p > F$		
Tillage practice		<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
N fertilizer		<0.001 ***	<0.001 ***	<0.001 ***	ns	<0.05 *	<0.001 ***
Tillage × N fertilizer		<0.001 ***	ns	ns	<0.001 ***	<0.001 ***	ns

NT: No till without cover crops. NTcc: No till with cover crops. CT: Conventional tillage without cover crops. CTcc: Conventional tillage with cover crops. N0: no added N fertilization. N1: 161 kg N ha^{-1}. N2: 215 kg N ha^{-1}. AEN: N agronomic efficiency. PFPN: N partial factor productivity. NAR: N apparent recovery fraction. Soil management practices (CT and NT) and N fertilization (N0, N1 and N2) were subjected to variance analysis (Two-way ANOVA, with tillage practice with or without cc and N fertilization as the two main parameters). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter within a column are not significantly different. (*, *** = significant at 0.05, 0.001 probability level, respectively). ns = not significant.

2.3. *Effect of Tillage Treatment and N Fertilizer Rate on NNI*

An increase of NNI was observed both in 2014 and 2015 with increasing rates of N fertilization from N0 to N2 (Figure 4). An analysis of covariance ANCOVA test demonstrated that the slopes of the lines for CT, CTcc, NT and NTcc were significantly different only in 2015 ($p < 0.001$). Moreover, the y-intercepts for these four cultivation conditions were significantly different ($p < 0.01$, $p < 0.001$ in 2014 and 2015, respectively). Compared to CT, the y-intercepts were always higher under NT irrespective of the presence of cc. In addition, the presence of cc significantly increased NNI under both CT and NT conditions (Figure 4).

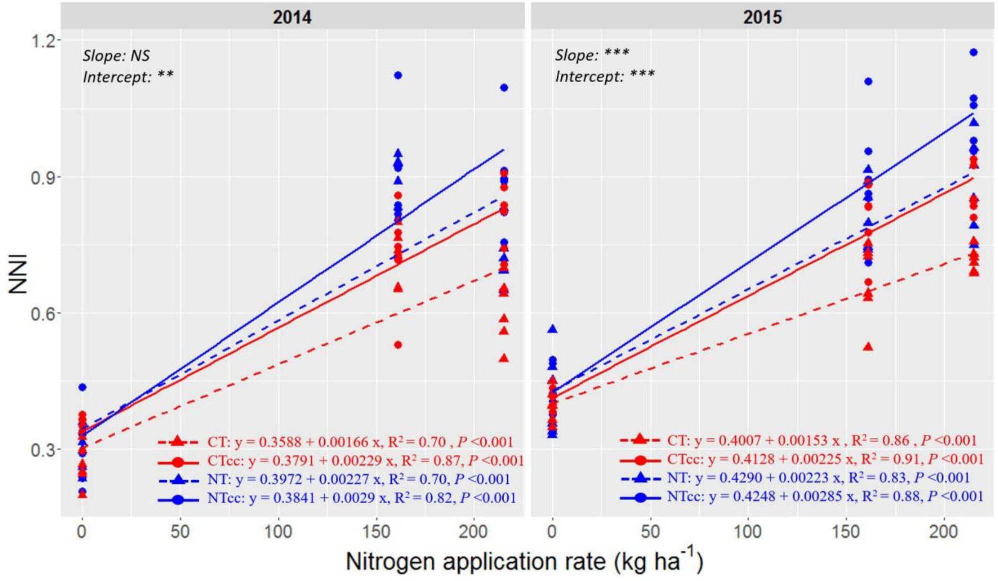

Figure 4. Nitrogen nutrition index (NNI) of wheat as a function of N application rate grown with four different tillage treatments in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. Slopes and intercepts obtained with the four tillage treatments were evaluated using analysis of covariance (ANCOVA). ** and *** = significant at 0.01, 0.001 probability level respectively, as indicated on the top left hand side of the figures.

When NNI and NUE were plotted again each other, a negative relationship was observed between the two agronomic traits. The differences between the two CT and NT conditions were similar to that observed for NNI alone. Over the two years of experimentation, the y-intercepts of CT, CTcc, NTcc and NT were significantly different ($p < 0.001$). Moreover, the slopes of the four regression lines were significantly different in 2015 ($p < 0.05$), but not in 2014. The slopes representing the negative linear function between NNI and NUE were higher in the absence of cc under conventional tillage (Figure 5).

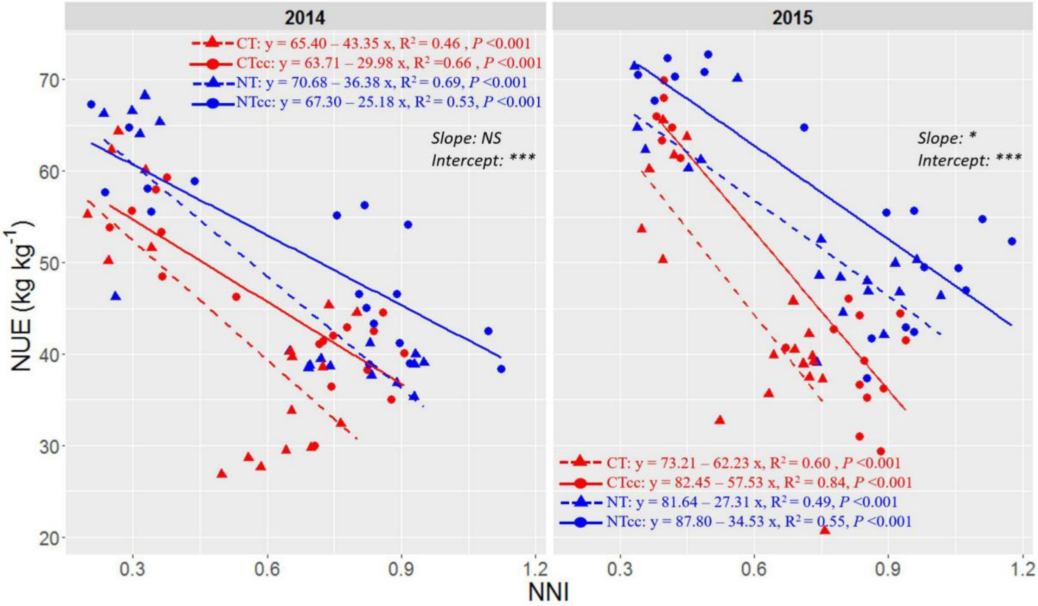

Figure 5. Nitrogen-use efficiency (NUE) of wheat as a function of N nutrition index (NNI) grown with four different tillage treatments in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. *Slopes* and *intercepts* obtained with the four tillage treatments were evaluated using analysis of covariance (ANCOVA). * and *** = significant at 0.05, 0.001 probability level respectively, as indicated on the top right hand side of the figures.

2.4. Correlation Analysis

Principal component analysis (PCA) was performed in order to obtain a visual representation of the correlations existing between agronomic traits (yield, N grain, aboveground biomass, total aboveground N, soil N, and NNI) and NUE traits (NUE, NUtE, AEN, PFPN, NAR and NRem). The first two axes of the PCA explained 80.2 % of the variance in all studied traits. Traits were separated into twelve groups namely CTccN0, CTccN1, CTccN2, CTN0, CTN1, CTN2, NTccN0, NTccN1, NTccN2, NTN0, NTN1 and NTN2 corresponding to the combined effect of the tillage system, the presence of cc and the amount of N fertilization. Axis 1 (71.1% of variance explained) allowed a separation of the N0 N fertilization treatment from both N1 and N2 N fertilization. NT and CT were clearly separated along Axis 2 (9.1% of variance explained). Cover crops (cc) conditions were not clearly separated along the two axes. NUE and NUtE were markedly higher when there was no added N fertilization (N0) and were highly correlated with the NT system. NRem, NNI, AEN and PFPN were higher under the NT system following treatment with either N1 or N2 N fertilizer (Figure 6).

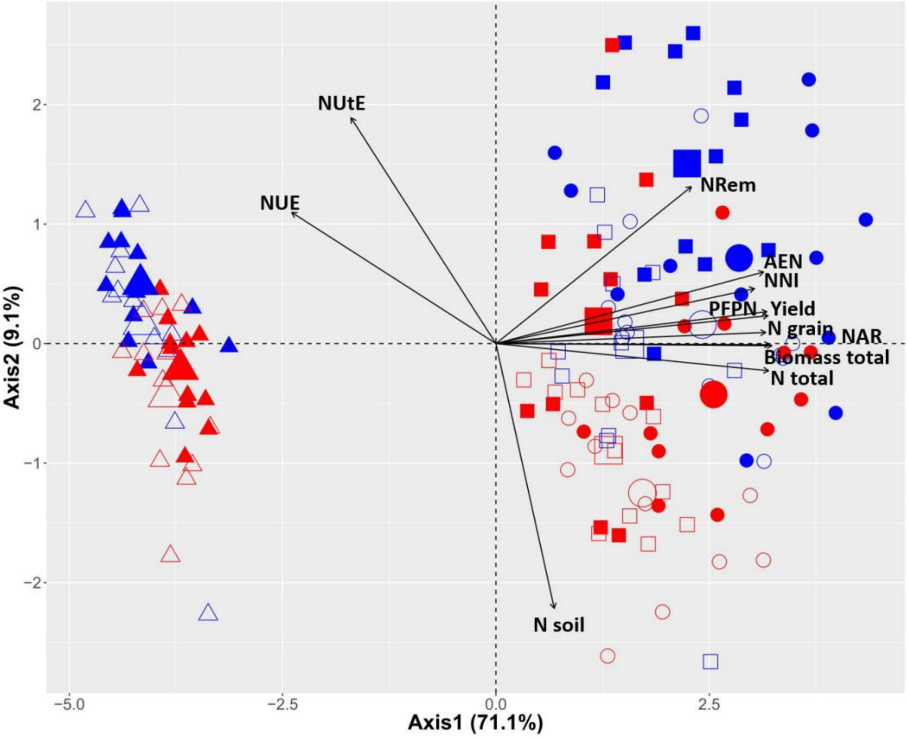

Figure 6. PCA analysis showing the correlations between agronomic traits and NUE traits according to the tillage conditions, the presence of cover crops and the level of N fertilization over two years. Diagrams are defined by the first two axes of the PCA of the different variables (*n* = 12); Axis1 (71.1% of variance explained) and Axis2 (9.1% of variance explained). NTcc: No till with cover crops. NT: No till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no added fertilization. N1: 161 kg N ha^{-1}. N2: 215 kg N ha^{-1}.

3. Discussion

A gradual increase in aboveground plant biomass, grain yield and grain N content was observed over the two years of field trials, thus validating the experimental design with regards to the rate of N fertilization. At the three levels of N fertilization both grain yield and grain N content were maintained under NT conditions compared to CT irrespective of the presence of cc. In a number of studies, it has been shown that in wheat, NT or reduced tillage can in some cases lead to an increase in grain yield in comparison to conventional agricultural systems [33–36]. In contrast, Machado et al. [37] found that over six years, the yield of spring wheat was on average 21% lower in NT soils, thus indicating that the impact of NT on grain production largely depends on the environmental conditions and on the experimental set up.

The two-year field experiment showed that for wheat, NT irrespective of the presence of cc, had a positive impact on a number of key NUE-related traits such as NUE, NUtE, AEN, PFPN and NAR in N1 for one of the two years. Following growth under NTcc and NT conditions, NUE traits were significantly higher than when grown using CT (Figures 1 and 2 and Table 2). These results are in agreement with those obtained by Soon et al. [38] and Chang et al. [39], who showed that for wheat, NUE was higher under NT compared to CT conditions (Figure 1). It should be noted that on some occasions with spring wheat, different results were obtained, when NUE was higher in tilled soils due to an increase in yield [11,40]. In this study, the effect of cc was variable between years, with positive effects on NUE-traits in certain tillage and N fertilization conditions (Figures 1–3 and Table 2). It has been reported previously that NUE was increased by cc under both NTcc and CTcc conditions, compared to NT and CT respectively (Figure 1). Such positive effects of the cc on NUE were attributed to a better N supply originating from the cc residues and to an improvement in the physical and chemical properties of the soil [41,42].

In many cases, it has been shown that NT had a negative impact on NUE, mostly because grain yield was reduced [11,40,43]. In other studies, it was found that NUE was increased under NT conditions, probably due to a reduction in plant N uptake. This is likely because the availability of N in the soil was lower, thus increasing the ratio of yield/N uptake [44,45]. In the present study, we found that NUE-related traits were higher under NT conditions, irrespective of the presence of cc. One possible explanation could be that the capacity of the plant to take up N at a high rate post-anthesis is maintained under NT conditions, thus enhancing NUE.

In addition, we found that remobilized N (NRem) was higher following growth under no-till (NTcc) compared to conventional till (CTcc) conditions, in the presence of cc in N1 over two years and in N2 only in one of the two years (Figure 3).

To our knowledge, the effect of tillage on the N Nutrition Index (NNI) has not previously been evaluated in wheat. The y-intercepts of the linear regression lines using the two tillage and cc systems as the four main variables were significantly different over the two years of experimentation, meaning that NNI was different under the four growth conditions. The slopes of the four linear regression lines were significantly different in 2015. The y-intercepts of the four tillage conditions were higher when there was no-tillage (NTcc and NT) prior to growth on the three rates of applied N fertilizer, compared to conventional tillage (CTcc and CT) (Figure 4). These results suggest that N is limiting at the early stages of plant growth following tillage, due to a faster growth rate of the shoots during this period. However, at maturity, both total aboveground plant N and grain N content were similar under NT and CT conditions. Stanislawska-Glubiak and Korzeniowska, [46] suggested that wheat plants grown under CT conditions tend to enter into their vegetative developmental phase earlier than plants cultivated under NT conditions.

To take into account any correlation that may exist between N uptake and crop growth, we examined if the relationship between NNI and NUE for each tillage treatment was linear (Figure 5). We observed that NUE was inversely proportional to NNI and that for a similar plant N status, NUE was higher following no-till treatment (NTcc and NT) compared to conventional tillage (CTcc and CT). Such a higher NUE under the two NT conditions, could be attributed to a better plant fitness during vegetative growth. Šíp et al. [36] suggested that during plant vegetative growth, the environmental conditions were more favorable for N uptake, due to a better soil moisture availability when tillage was reduced. Moreover, Maltas et al. [47] reported that when the soils were converted from CT to NT in the presence of cc, N mineralization increased over the conversion period, thus increasing the availability of soil N [48].

PCA analysis allowed a visualization of the correlations between agronomic traits and NUE traits, as well as their relationship with the tillage system and the level of N fertilization (Figure 6). The first axis clearly separated the N0 plot from the N1 and N2 plots of N fertilization. The second axis mainly separated NT from CT plots. NUE and NUtE were considerably higher under NT conditions, when the plants were grown following no added N fertilization (N0). Similarly, NRem, NNI, PFPN and AEN were higher under NT conditions, when the plants were grown following either N1 or N2 N fertilization. Such an analysis thus confirms that the NT system had a positive impact both on NNI and NUE traits. Moreover, tillage had no effect on grain yield and grain N in both years. Our results indicate that NUE improvement can be obtained by combining NT and the use of cc.

4. Materials and Methods

4.1. Site Description and Experimental Design

Field experiments were conducted in La Woestyne, northern France (50°44′ N, 2°22′ E, 40 m above sea level). Soil physical and chemical characteristics are provided in Habbib et al. [26]. Weather-related parameters for this area were as follows: average annual precipitation 675 mm, average annual temperature 10.5 °C. The field was cultivated using a chisel plough and rotary power system until 2010, when the experiment was initiated. The experimental field was split into twelve treatments with three replicated plots per treatment placed randomly: three different N fertilization regimes (no added N (N0), 161 kg N ha^{-1} (N1) and 215 kg N ha^{-1} (N2)); four tillage/cover crop (cc) systems: no-till with (NTcc) or without (NT) cover crops and conventional tillage with (CTcc) or without (CT)) cover crops. The individual plot size was 7 m × 8 m for each treatment.

In 2014 and 2015 (4 and 5 years after the beginning of the field experiment, respectively), wheat (*Triticum aestivum* cv. BTH intensive EXPERT, Syngenta, Switzerland) was sown in October and then plant samples were collected from each plot (see Section 4.3 for details). Two different field plots were used in 2014 and 2015. The crop rotation preceding wheat cultivation in 2014, consisted of maize (*Zea mays* L.) in 2010, followed by wheat (*Triticum aestivum* L.) in 2011, flax (*Linum usitatissimum* L.) in 2012 and beet (*Beta vulgaris* L.) in 2013. In the wheat cultivation performed in 2015, the crop rotation consisted of pea (*Pisum sativum* L.) in 2010, followed by maize (*Zea mays* L.) in 2011, wheat (*Triticum aestivum* L.) in 2012, flax (*Linum usitatissimum* L.) in 2013 and beet (*Beta vulgaris* L.) in 2014.

The conventional tillage in CTcc and CT plots was performed using the mouldboard ploughing technique to 30 cm depth, followed by the passing of a rotating harrow (Kuhn, France) for shallow tillage. Cover crops (cc) were planted each year in August following 2010, after harvesting the main crop and were terminated before the sowing of the main crop. Before the sowing of the main crop, cc residues were buried in CTcc plots and left on the soil surface in NTcc plots. The cc consisted of a mixture of three legumes and three non-legume species, which were sown as follows: 400 seeds m^{-2} of Egyptian clover (*Trifolium alexandrinum* L.), 30 seeds m^{-2} of faba bean (*Vicia faba* L.), 50 seeds m^{-2} of vetch (*Vicia sativa* L.), 80 seeds m^{-2} of flax (*Linum usitatissimum* L.), 200 seeds m^{-2} of phacelia (*Phacelia tanacetifolia* Benth.), 60 seeds m^{-2} of oats (*Avena sativa* L.).

The amount of N fertilizer applied for the N2 treatment was determined according to the N budget method for winter wheat, based on the predictive balance-sheet method (Software Azobil, INRA, Laon, France)[49], using the following formula:

$$B + Rf = Ri + Mn + X \tag{1}$$

where B is the N required by the crop, Rf is the residual soil mineral N content at harvest in the previous year, Ri is the soil mineral N readily available at a determined depth of soil before wheat planting, Mn is the N mineralization potential during the growing season, and X is the N fertilizer rate. Mn results from the sum of the net mineralization from SOM, including the humic substances (Mh in Azobil), the N supply from previous crop residues (Mhr in Azobil), and the N supply from organic manures (Ma in Azobil), all three N sources originating from the decomposition of organic matter that is further mineralized into inorganic N. All of the terms of the equation are expressed in kg N ha^{-1}.

The N1 treatment represents an economic N input. The amount of N fertilizer applied under N1 conditions was calculated as N2 minus 25%. The N fertilizer was composed of 50% urea, 25% ammonium, 25% nitrate applied in a liquid form on the soil surface, through broadcast applications at nightfall. Under these conditions of application, it was assumed that N volatilization was negligible.

4.2. Soil Sampling and Chemical Analysis

Prior to the first application of N fertilizer in March 2014 and 2015, six 30 cm deep soil cores were randomly collected using a 2 cm diameter auger from each of the three replicate plots, for each treatment. The six soil cores from each replicate plot were combined together as a single composite sample. Soil samples were then air dried, grounded, and sieved using a 2 mm mesh and divided into two parts for total soil N and residual N analysis. For soil total N measurements, the sieved soil samples were dried in an oven at 45 °C for 48 h and ground using a MM 400 mill (Retsch, Germany). Soil total N concentration (expressed as % of dry soil), was quantified using the combustion method of Dumas [50] using a Flash EA 1112 elemental analyzer, Thermo Electron, Germany.

4.3. Crop Sampling and Plant Analysis

Wheat seeds were sown in October in rows 12.5 cm apart using an AS 400 drill (Alpego, Italy). At anthesis in May and at crop maturity in July, 6 rows of plants of 1 m in length (6 replicates for each of the three plots per treatment) were sampled from each treatment. The shoots were clipped at the ground level and threshed to separate the grains for measurements of yield. Shoots and grains were dried at 60 °C for 3 days, weighed and ground using a ZM 200 mill (Retsch, Germany), to obtain a fine powder (0.75 mm particles). Grain and straw N concentrations were determined on the six replicates for each of the three plots using the same elemental analyzer as for total N soil concentration.

The critical N dilution curve is related to aboveground biomass of a crop and crop N uptake during the vegetative growth [51]. The NNI is calculated as follows [21]:

$$\mathrm{NNI} = Nms/Nc \tag{2}$$

where, Nms corresponds to the % N concentration of the above ground plant material at flowering, and Nc to the critical plant N concentration defined as the minimum N concentration needed for maximum growth rate, calculated as

$$Nc = a \cdot (DM)^{-b} \tag{3}$$

where, DM corresponds to the aboveground dry matter produced (Mg ha^{-1}). For the reference curve of wheat: a = 5.35 and b = 0.442 [21].

Traits related to NUE were calculated according to Moll et al. [52]; Dobermann, [15]; López-Bellido et al. [12] and Huggins and Pan, [11] using the following formulae:

$$\begin{aligned}\mathrm{NUE}\ (\mathrm{kg\ kg^{-1}}) = Gy/\mathrm{N\ supply\ in\ fertilized\ plots}\\ Gy0/\mathrm{N\ supply\ in\ control\ plots}\end{aligned} \tag{4}$$

$$\begin{aligned}\mathrm{NUtE}\ (\mathrm{kg\ kg^{-1}}) = Gy/Nt\ \mathrm{supply\ in\ fertilized\ plots}\\ Gy0/Nt0\ \mathrm{supply\ in\ control\ plots}\end{aligned} \tag{5}$$

$$\mathrm{AEN}\ (\mathrm{kg\ kg^{-1}}) = (Gy - Gy0)/X \tag{6}$$

$$\mathrm{PFPN}\ (\mathrm{kg\ kg^{-1}}) = Gy/X \tag{7}$$

$$\mathrm{NAR}\ (\%) = (Nt - Nt0)/X \tag{8}$$

where, Gy corresponds to grain yield in fertilized plots (kg ha^{-1}), $Gy0$ to grain yield in the control unfertilized plots (kg ha^{-1}), Nt to total aboveground plant N at maturity in fertilized plots (kg ha^{-1}), $Nt0$ to total aboveground plant N at maturity in the control plots (kg ha^{-1}), c to the amount of N applied to the soil (kg ha^{-1}). Both the N supply to the soil and the N available to the crop are expressed in kg kg^{-1}. The available N corresponds to the sum of applied N fertilizer and total plant N uptake in non-fertilized plots for each tillage system [26].

To measure the amount of N remobilized from vegetative to reproductive organs after anthesis (NRem), the following equations were used according to the methods described by Cox et al. [53]; Beheshti and Behboodi, [54] and Masoni et al. [55]:

$$\begin{aligned}\mathrm{NRem}\ (\mathrm{g\ plant^{-1}}) = \mathrm{N\ content\ of\ the\ aboveground\ plant\ material\ at\ anthesis}\\ - \mathrm{N\ content\ of\ leaves,\ stem\ and\ chaff\ at\ maturity}\end{aligned} \tag{9}$$

4.4. Statistical Analyses

All statistical analyses were performed using R statistical software (version 3.3.0, 2015, Vienna, Austria) [56]. Data were subjected to variance analysis (Two-way ANOVA), using soil management (CT with or without cc and NT with or without cc) as the main parameter and the level of N application (N0, N1, N2) as the second parameter. All explanatory variables were examined for normality using the Shapiro-Wilk test and for homogeneity of variances using the Bartlett test (Agricolae package). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level (Agricolae package). Linear regression was performed to evaluate the slope between NNI and N fertilization application for each tillage treatment. To account for the allometry between N uptake and crop growth, a linear model was constructed between NNI and NUE for each tillage treatment. Analysis of covariance (ANCOVA) (Stats package), was applied in order to compare slopes and intercepts obtained with four tillage/cc conditions (NTcc, NT, CTcc and CT). Principal component analysis (PCA) (ade4 package) was also carried out to visualize relationships existing among agronomic traits (yield, N grain, aboveground plant biomass, total aboveground plant N, N soil and NNI) and NUE traits (NUE, NUtE, AEN, PFPN, NAR and NRem).

5. Conclusions

The impact of no-till(NT) on both NUE and grain yield of wheat still needs to be fully assessed, due to the number of cases of negative effects on these two traits, that have been reported previously (see Discussion). In the present study, we showed that five years after conversion to a no-till system, similar yields were obtained in comparison to continuous conventional tillage conditions. Under no-till conditions, NUE, NUtE and other NUE-related traits such as AEN, PFPN, NAR and NNI were increased at least in one year of experimentation. However, we observed that in some cases the increase in these traits did not occur at all three levels of N fertilization. Taken together, these results demonstrated that the positive effects of no-till on plant performance and NUE-related traits strongly depended on the presence of cover crops (cc). In addition, these positive effects also depended on the level of N fertilization and were not necessarily observed from one year to the other. Thus, our findings could partly explain why contrasting results have been reported in the literature. Both the design of the field trial and the environmental conditions, notably N fertilization, appear to be key parameters for obtaining reproducible results over several years. Nevertheless, our study strongly suggests that the use of a continuous no-till system with cc is a promising strategy for sustainable wheat production based on the reduction of N fertilizer use, without any yield penalty.

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