J. Crop Sci. Biotech. 2020 (January) 23 (1) : 39 ~ 46
DOI No. 10.1007/s12892-019-0122-0

RESEARCH ARTICLE

In Winter Wheat (*Triticum Aestivum* L.), No-Till Improves Photosynthetic Nitrogen and Water-Use Efficiency

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Received: April 26, 2019 / Revised: May 27, 2019 / Accepted: May 29, 2019

Abstract

To evaluate the combined effect of different agricultural practices on photosynthetic nitrogen and water-use efficiency, winter wheat was grown in the field under tillage and no-till conditions, with and without cover crops under low and high nitrogen fertilization inputs. Leaf physiological traits, such as the rate of photosynthesis, stomatal conductance, the rate of transpiration, the chlorophyll content index, the leaf area ratio, and specific leaf area were used as indicators representative of nitrogen and water-use efficiency. Six years after conversion to no-till, in the presence and in the absence of cover crops, significant increases in photosynthetic water-use efficiency and soil water content were observed both under low and high nitrogen fertilization input. Moreover, we observed that photosynthetic nitrogen-use efficiency, the rate of photosynthesis and specific leaf area were higher in the absence of tilling than in the presence of tilling. Thus, agronomic practices based on continuous no-till appear to be promising for increasing both photosynthetic nitrogen- and water-use efficiency in winter wheat.

Key words : Cover crops, nitrogen, no-till, photosynthesis, water, winter wheat

Introduction

Crop productivity is mainly determined by both the availability and the efficient utilization of limiting soil resources such as water and nitrogen (N) (Cossani et al. 2012). Photosynthetic nitrogen-use efficiency (PNUE) and photosynthetic water-use efficiency (PWUE) are two key agronomic traits that are commonly used to estimate the efficiency with which these two resources are used to ensure optimal plant growth and development (Blankenagel et al. 2018; Castellanos et al. 2005; Guo et al. 2016).

PNUE is defined as the ratio of photosynthesis rate to leaf N (Anand et al. 2007). Thus, a high plant growth rate is generally associated with a high PNUE (Dinh et al. 2017; Hikosaka 2004).

Leaf water-use efficiency (WUE), defined as the ratio of carbon (C) assimilation to water consumption, is one of the parameters commonly used to estimate plant water-use efficiency during its developmental cycle (Flexas et al. 2013; Wu and Bao 2012).

Currently, improving both PNUE and PWUE in crops is a prerequisite for maintaining or even enhancing crop productivity in order to feed the constantly growing world population in a changing environment (Easterling et al. 2007; Hirel et al. 2011). Among the various agricultural practices currently used by farmers, conservation agriculture based on no-till (NT) is increasingly used to ensure both crop productivity and optimal soil mineral resource recovery (Dawson et al. 2008) while maintaining both soil organic matter (SOM) and water contents (Awale et al. 2013; Christopher et al. 2009; Dalal et al. 2011; Dimassi et al. 2013). The absence of tillage maintains soil moisture because the total pore space and the relationship between macro- and micro-pores is not altered, as occurs in tilled soils (Josa March et al. 2010). Consequently, maintaining soil moisture under no-till conditions is beneficial

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to a crop such as wheat, notably after anthesis (Thomas et al. 2007), a period during which both N uptake and remobilization are maintained to ensure optimal grain filling even when there is a shortage of water (Bahrani et al. 2011).

A large number of studies have been conducted in order to develop alternative N fertilizer management practices for simultaneously increasing PNUE and PWUE in wheat (Dawson et al. 2008; Dinh et al. 2017; López-Bellido et al. 2005; Shangguan et al. 2000). However, the efficiency by which N and water are used in different cropping systems has only been evaluated in a limited number of studies (Delogu et al. 1998; Wu and Bao 2012; Zheng et al. 2014). Moreover, in winter wheat the impact of no-till has only been investigated for PWUE (Hou et al. 2013). In the present study, wheat plants were grown under tillage and no-till conditions with and without cover crops (cc) in order to evaluate the combined effect of tilling and N fertilization on PNUE and PWUE. Leaf WUE and PNUE are generic agronomic traits resulting from the combined effect of individual leaf physiological traits. For example, WUE increases when leaf stomatal conductance (g_s) and transpiration rate (E), reflecting the of carbon dioxide (CO_2) entering and the rate of water vapor exiting through the stomata, are reduced (Baker et al. 2007; Sharkey et al. 2007). Irrespective of the strong relationship existing between the rate of photosynthesis (A_{sat}) and the leaf N content, their ratio corresponding to PNUE, is strongly correlated with the specific leaf area (SLA) (Poorter and Evans 1998). As such, in comparison to plants with low SLAs, plants with high-SLAs plants tend to have a higher PNUE. Therefore, leaf physiological markers, including A_{sat}, g_s, E, and SLA were used to compare the PNUE and WUE of wheat plants grown in tilled and non-tilled soils. In addition, the leaf area ratio (LAR), corresponding to the photosynthetic surface area per unit of dry matter (DM) of a plant, was also measured to evaluate the efficiency with which wheat plants deployed their photosynthetic capacity in ploughed and non-ploughed soils.

Materials and Methods

Site description and experimental design

Field experiments were conducted in Woestyne, northern France (50°44'N, 2°22'E, 40 m above sea level). Physical and chemical soil characteristics of this area are described in Habbib et al. (2016). Weather-related parameters for this area are described in Habbib et al. (2017). The field was managed under a chisel plough and rotary power system until 2010, when the experiment was initiated.

The experimental field contained of eight different treatments with four replicated plots per treatment placed randomly. The eight different treatments were two N fertilization regimes [no added N (N0) and 215 kg N ha^{-1} (NX)] applied to four tillage/cover crop (cc) cultivation systems including no-till with cc (NTcc) or without cc (NT) and conventional tillage with cc (CTcc) or without cc (CT). The individual plot size was 7 × 8 m for each treatment. The conventional tillage in the CTcc and CT plots was performed using the mouldboard ploughing technique to a depth of 30 cm, followed by the passing of a rotating harrow (Kuhn SA, Saverne, France) for shallow tillage.

In 2016, (6 years after the beginning of the field experiment), wheat (*Triticum aestivum* cv. BTH intensive EXPERT, Syngenta, Switzerland) was sown in October and then plant samples were collected from each plot (see Section 2.2 and 2.3 for details). The crop rotation preceding wheat cultivation in 2016 consisted of wheat (*Triticum aestivum* L.) in 2010 followed by pea (*Pisum sativum* L.) in 2011, maize *(Zea mays* L.) in 2012*,* wheat (*Triticum aestivum*) in 2013, flax (*Linum usitatissimum* L.) in 2014, and sugar beet (*Beta vulgaris* L.) in 2015.

Before sowing of the main crop, cover crop residues were buried in the CTcc plots and left on the soil surface in NTcc plots. The cc consisted of a mixture of legume and non-legume species as previously described in Habbib et al. 2017. The amounts of N fertilizer applied under NX conditions (215 kg N ha^{-1}) were determined according to the N budget method for maize (Machet et al. 1990), based on the predictive balance-sheet method (Software Azobil, INRA, Laon, France) as previously described (Habbib et al. 2016, 2017)

The N fertilizer was composed of 50% urea, 25% ammonium, and 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.

Gas exchange measurements

During the anthesis period (between late May and early June), the flag leaves of four different plants randomly selected in each of the four plots used per treatment (NTcc, NT, CTcc, and CT in N0 and in NX) (see section 2.1) were used for gas exchange measurements. Gas exchange parameters were measured using a LI-6400XT portable photosynthesis system (LI-COR Environmental, Cambridge, UK) equipped with CO_2 and temperature control modules a 1*1 cm sample chamber and a red-blue LED light source (LI-COR Biosciences, Lincoln, Nebraska 68504, USA).

Before performing gas exchange measurements, the flag leaf was acclimated in the LI-COR chamber for at least 10 min at 21 ± 1.2°C under ambient humidity with a CO_2 concentration of 400 ppm and a photosynthetic photon flux density (PPFD) of 1100 µmol m^{2} s^{-1}, conditions under which photosynthesis is nearly saturated. Photosynthetic activity was represented by the light-saturated rate of net CO_2 fixation (Asat).

At the leaf scale, PWUE corresponds to the amount of C assimilated per unit of leaf area, per unit of time and per cost unit of water. Intrinsic water-use efficiency (WUEint) was calculated as the ratio of light-saturated net CO_2 assimilation (A_{sat}, µmol m^{-2} s^{-1}) to stomatal conductance (g_s, µmol m^{-2} s^{-1}) (Ehleringer 1993). Instantaneous water-use efficiency (WUEins, transpiration efficiency at the leaf level) was calculated as the ratio of CO_2 assimilation (A_{sat}, μmol m^{-2} s^{-1}) to transpiration rate (E, mmol m^{-2} s^{-1}) per unit of leaf area (Medrano et al. 2015).

Plant sampling and analysis

Following the gas exchange experiments, the same four different wheat plants were harvested to measure total leaf area and above ground DM. Flag leaves were scanned using an HP LaserJet Pro scanner. The images were then digitized using the ImageJ software (Schneider et al. 2012) to calculate total leaf area.

The flag leaves and the remaining shoots were then dried separately in an oven for 48 h at 70°C. The specific leaf area (SLA, cm^2 g^{-1}) was calculated as the total leaf area divided by the leaf DM. The leaf area ratio (LAR, cm^2 g^{-1}) was calculated as the total leaf area divided by the whole plant DM. After drying, flag leaves were finally ground in a Retsch mill (Retsch zm200, Haan, Germany) to obtain a fine powder (0.75 mm particles). Leaf N content expressed in % (Nmass) was quantified using the combustion method of Dumas (Dumas 1831) using a Flash EA 1112 elemental analyzer (Thermo Electron, German). Photosynthetic N-use efficiency (PNUE) was calculated as the ratio of A_{sat} to leaf N content, both expressed per unit leaf area (Lopes and Araus 2006). The chlorophyll content index (CCI) was measured on the flag leaves, which had already been used to perform gas exchange experiments, using a portable chlorophyll meter (CCM200, Opti-Sciences, Inc., Hudson, USA). The CCI is an arbitrary unit obtained by calculating the ratio of the optical absorbance measured at 655 and 940 nm.

Soil water content

At the same time as the gas exchange measurements were conducted, the soil water content (SWC, expressed in %) at a depth of 20 cm was determined in each treatment using a soil moisture metre (FieldScout TDR 100, USA).

Statistical analyses

All statistical analyses were performed using R © software version 3.3.0. Data were subjected to non-parametric Kruskal–Wallis one-way analysis of variance (*H*-value) followed by a Dunn's post hoc test whenever significant (Agricolae package) (De Mendiburu 2015). Correlations between photosynthesis efficiency traits (PNUE, WUEins and WUEint), SWC, and leaf phenotypic and physiological traits (SLA, LAR, Nmass, A_{sat}, gs, E, and CCI) were calculated using a Spearman correlation coefficient at $P < 0.05$ (Hmisc package) (Harrell 2015). Principal component analysis (PCA) (ade4 package) (Dray et al. 2015) was also carried out to visualize the relationships existing between our traits to determine how the photosynthesis efficiency traits were related to the environmental and physiological variables between treatments (NTcc, NT, CTcc, and CT in N0 and NX).

Results

Effect of tillage, cover crops and N fertilization on WUEint, WUEins and PNUE

The two parameters WUEint and WUEins, representative of the photosynthetic water-use efficiency (PWUE), were significantly modified ($P < 0.01$) depending on the tilling, the level of N fertilization and the presence of cover crops (cc) (Fig. 1). In Fig. 1A, WUEint was markedly higher (40%) under NTcc than under CTcc only under high N fertilization input (NX). When no N fertilizers were applied to the field (N0), WUEint was not significantly different regardless of the tilling conditions (CT and NT). In N0, cc had a low (<10%) but positive impact on WUEint which was significantly higher in the presence of cc only under CT (CTcc) than under the other conditions. Similar differences were observed for WUEins. In addition, a 30% increase in WUEins was also observed under CT in NX with cc. In N0 WUEins was

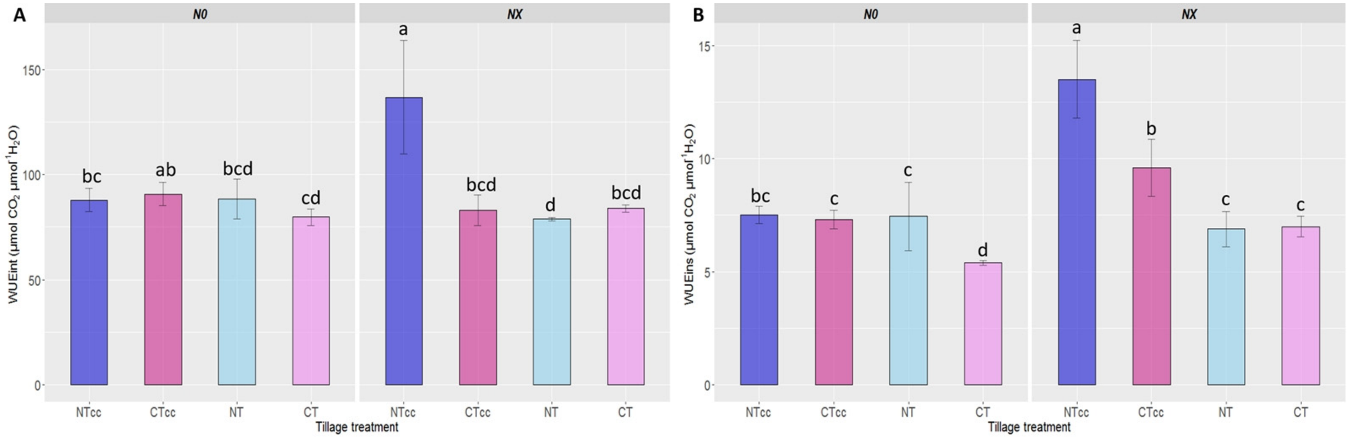

Fig. 1. Impact of tillage and nitrogen fertilization on WUEint (A) and WUEins (B): NTcc = No Till with cover crops (cc), NT= no till without cover crops, CTcc = conventional tillage with cc and CT = conventional tillage without cc. N0 = no fertilization and NX = optimal N fertilization. Data for each parameter were subjected to a Kruskal–Wallis one-way analysis of variance. Treatment means were compared using Dunn's post hoc test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

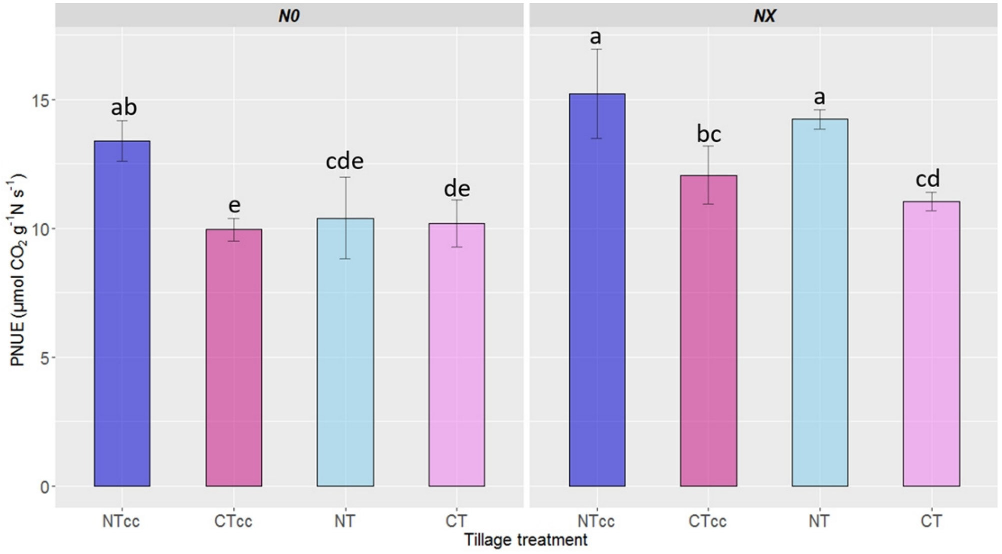

Fig. 2. Impact of tillage and nitrogen fertilization on PNUE: NTcc = No Till with cover crops (cc), NT= no till without cover crops, CTcc = conventional tillage with cc and CT = conventional tillage without cc. N0 = no fertilization and NX = N fertilization. Data for each parameter were subjected to Kruskal–Wallis one-way analysis of variance. Treatment means were compared using Dunn's post hoc test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

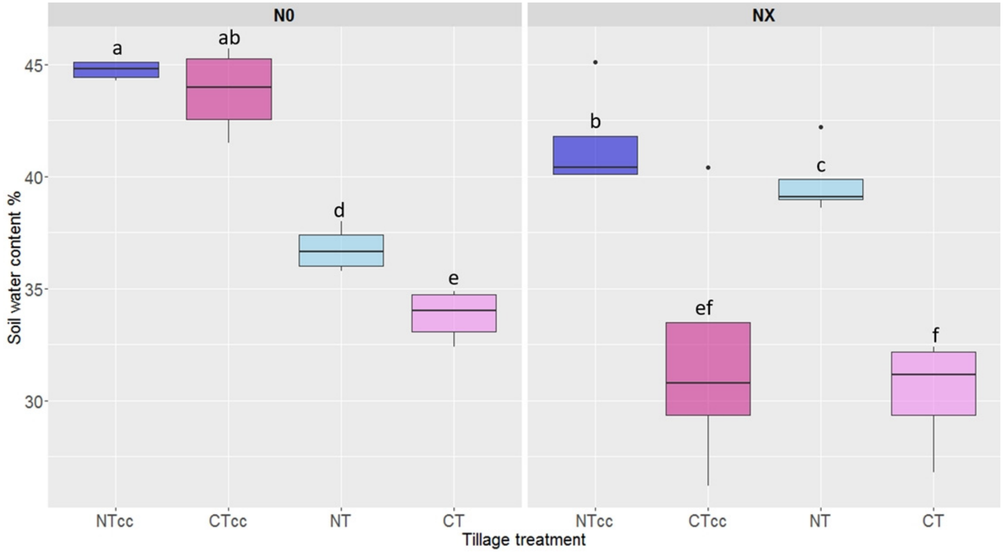

Fig. 3. Impact of tillage and nitrogen fertilization on SWC: NTcc = No Till with cover crops (cc), NT= no till without cover crops, CTcc = conventional tillage with cc and CT = conventional tillage without cc. N0 = no fertilization and NX = N fertilization. Data for each parameter were subjected to Kruskal–Wallis one-way analysis of variance. Treatment means were compared using Dunn's post hoc test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

higher under NT conditions in the absence of cc than under the other conditions.

In general, tillage had a significant negative impact on photosynthetic nitrogen-use efficiency (PNUE) at the two levels of N fertilization and in the presence of cc (Fig. 2). In addition, we observed that PNUE was higher in NX under NT conditions irrespective of the presence of cc than under the other conditions.

Effect of tillage, cover crops and N fertilization on the soil water content

As shown in Fig. 3, the highest decrease in the soil water content (SWC) was observed in the absence of cc in N0 irrespective of the tilling conditions ($P < 0.001$). In comparison to N0, in NX the SWC was on average much lower with no or limited differences between the plots cultivated in the presence or absence of cc. However, under the NX fertilization regime SWC was markedly decreased in the ploughed soil

Table 1. Impact of tilling, cover crops and nitrogen fertilization on wheat leaf physiological traits.

Source of variance		Leaf phenotypic and physiological traits						
N fertilizer	Tillage	Asat	gs	E	CCI	SLA	LAR	Nmass
N0	NTcc	15.06 ± 0.75 d	0.113 ± 0.002 a	1.41 ± 0.05 d	11.09 ± 1.08 c	176.22 ± 10.22 bc	3.08 ± 0.31 abc	1.99 ± 0.15 c
	CTcc	10.22 ± 0.47 e	0.172 ± 0.005 b	2.03 ± 0.18 bc	9.57 ± 1.18 cd	169.53 ± 4.17 d	2.88 ± 0.57 bc	1.74 ± 0.09 d
	NT	9.70 ± 1.40 e	0.118 ± 0.027 a	1.62 ± 0.51 bcd	8.86 ± 0.71 d	186.3 ± 5.05 ab	2.87 ± 0.26 c	1.74 ± 0.07 d
	CT	10.87 ± 0.85 e	0.137 ± 0.011 a	2.02 ± 0.17 bc	9.46 ± 1.50 cd	165.45 ± 4.04 d	1.87 ± 0.25 d	1.77 ± 0.03 d
NX	NTcc	21.05 ± 1.23 a	0.171 ± 0.033 b	1.64 ± 0.25 cd	25.45 ± 1.59 b	195.71 ± 16.38 ab	3.80 ± 0.84 ab	2.72 ± 0.05 ab
	CTcc	20.94 ± 2.51 ab	0.259 ± 0.045 c	2.29 ± 0.45 b	32.22 ± 3.03 ab	170.99 ± 14.12 cd	3.77 ± 0.52 ab	2.91 ± 0.12 a
	NT	19.34 ± 0.53 bc	0.246 ± 0.007 c	2.91 ± 0.33 a	34.34 ± 1.86 a	198.21 ± 5.56 a	3.67 ± 0.24 a	2.70 ± 0.14 b
	CT	18.43 ± 0.43 c	0.220 ± 0.008 c	2.66 ± 0.14 a	30.66 ± 2.95 a	170.37 ± 4.16 cd	3.15 ± 0.24 abc	2.85 ± 0.08 ab
	H	40.457 ***	36.679 ***	29.590 ***	40.059 ***	24.808 ***	22.583 **	38,658 ***

N0 = no fertilization, NX = N fertilization, NTcc = no-till with cover crops, CTcc= conventional tillage with cover crops, NT = no-till without cover crops, CTcc= conventional tillage without cover crops. A_{sat} = photosynthetic activity, g_s = stomatal conductance, E = transpiration rate, CCI= chlorophyll content index, SLA= specific leaf area, LAR = leaf area ratio, and Nmas = leaf N content%. *H*: value of the Kruskal–Wallis test with its significance in brackets (*, **, and *** = significant at 0.05, 0.01, and 0.001 probability level, respectively). Letters give the result of a Dunn's post hoc test at a 95% family-wise confidence level. Means sharing the same letter are not significantly different.

(CT). In N0 the SWC was also higher under NT conditions in the absence of cc than under the other conditions.

Effect of tillage, cover crops, and N fertilization on leaf physiological marker traits

The impact of tillage, N fertilization, and the presence of cc on representative leaf physiological traits, including the phostosynthetic activity (A_{sat}), stomatal conductance (g_s), transpiration rate (E), chlorophyll content index (CCI), specific leaf area (SLA), leaf area ratio (LAR), and the leaf N content (Nmass), are presented in Table 1. Compared to non-fertilized plots (N0), the plots fertilized with mineral N (NX) had higher Asat, gs, CCI, LAR, and Nmass. Tillage had a significant negative impact on A_{sat} under N0 only in the presence of cc. In NX, both in the absence or presence of cc, A_{sat} was not markedly modified. In the presence of cc, gs was higher in tilled plots than in no-till plots both in N0 and in NX. Tillage had a significant effect on E meaning that transpiration was higher in CTcc than in NTcc irrespective of the N fertilization conditions. Both in N0 and in NX, the CCI was not markedly modified in the different tilling and cover cropping conditions. No-till increased SLA under both N0 and NX conditions both in the presence and absence of cc. In N0, an increase in LAR was observed only under NT conditions in the absence of cc. Tillage did not modify leaf Nmass in N0 or in NX, except in the presence of cc in N0.

Correlation studies

Spearman correlations between WUEins, WUEint, and PNUE and the corresponding phenotypic and physiological marker traits are presented in Fig. 4. WUEint was significantly and positively correlated with SWC (0.4) and LAR (0.2) and negatively correlated with gs (-0.34) and E (-0.51). WUEins was significantly and positively correlated with CCI (0.25), LAR (0.38) and A_{sat} (0.38) and negatively correlated with E (-0.54). PNUE was significantly and positively correlated with CCI (0.45), SLA (0.54), LAR (0.43), Asat (0.67), gs (0.55), and E (0.45) Gs (0.55) and Nmass (0.3).

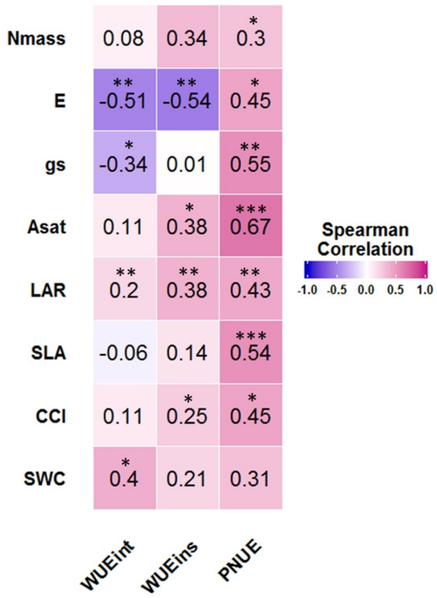

Fig. 4. Spearman correlation coefficient *r* between WUEins, WUEint, PNUE, SWC, and leaf physiological traits. (*, ** and ***= significant at 0.05, 0.01, and 0.001 probability level, respectively).

A PCA analysis was then performed in order to obtain a visual representation of the correlations among WUEins, WUEint, PNUE, SWC, and the different leaf phenotypic and physiological traits according to the tillage system and the level of N fertilization (Fig. 5). The first two axes of the PCA explained 67.5% of the variation when the eight different variables corresponding to the measured traits and the different plant growth conditions were considered.

For axis.1 (40.6% of the variance explained) there was a

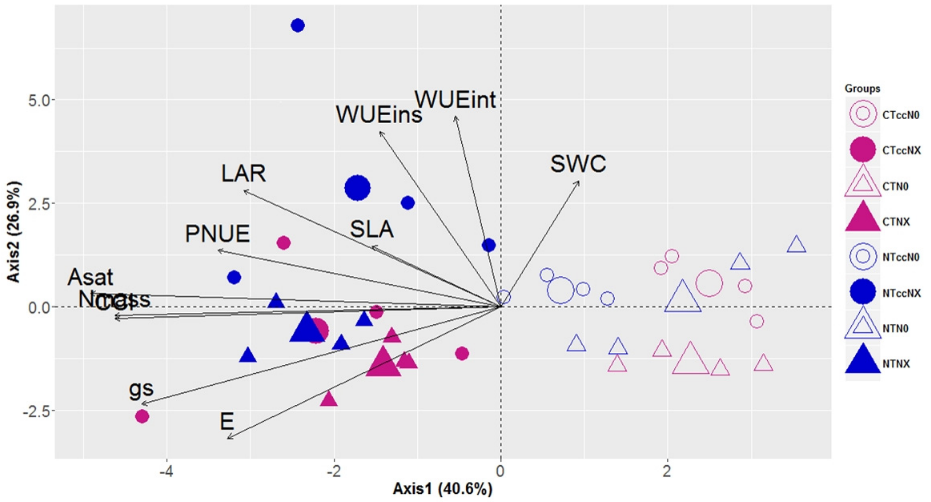

Fig. 5. PCA analysis showing the correlations between WUEins, WUEint, PNUE, SWC, and leaf phenotypic and physiological traits (SLA, LAR, Nmass, A_{sat}, gs, E, and CCI) according to the tilling and N fertilization conditions. Diagrams were defined by the first two axes of the PCA of the variables (n=4). Axis 1 (40.6% of the variance explained) and Axis 2 (26.9% of the variance explained). NTccN0 = no-till with cover crops without N fertilization, NTN0 = no-till without cover crops without N fertilization, CTccN0 = conventional tillage with cover crops without N fertilization, CTN0 = conventional tillage without cover crops without N fertilization, NTccNX = no-till with cover crops and N fertilization, NTNX= no-till without cover crops with N fertilization, CTccNX = conventional tillage with cover crops with N fertilization and CTNX = Conventional tillage without cover crops with N fertilization. The repartition of the measured traits within the four areas delimited by the two different axes are indicated by arrows.

positive correlation only with SWC, and a negative correlation with all the other traits. SWC, WUEint, WUEins, PNUE, SLA, LAR, and A_{sat} were strongly and positively correlated along axis.2 (26.9% of the variance explained). A negative correlation was found for CCI, g_s and E, which were grouped along Axis.2. Irrespective of the tillage and the cover cropping system, the two N fertilization, conditions were separated along axis 1. In the NX cluster, NTcc and CTcc were separated along axis 2. The three photosynthesis efficiency traits (A_{sat}, LAR, and SLA) and all the other physiological leaf traits were higher under the NX conditions irrespective of the tillage and the cover cropping system than under the other conditions. WUEint, WUEins, PNUE, and the two leaf traits LAR and SLA were markedly higher under NTcc than under CTcc under NX conditions.

Discussion

A field experiment was conducted in order to determine whether winter wheat uses N and water more efficiently when no-till was used instead of conventional tillage. Whether the presence of cover crops had any influence on the use of the two major soil limiting resources was also investigated since the combined effect of tilling and cover cropping on PNUE and PWUE has never been thoroughly investigated.

In the presence of cc, a higher WUEint (one of the two main components of PWUE reflecting the capacity of the leaf to assimilate CO_2 as a function of transpiration) was observed under no-till (NTcc) than under continuous till conditions (CTcc). The other component of PWUE, WUEins was also found to be higher under NT conditions even in the absence of cc than under the other conditions. These results demonstrate that a higher photosynthetic C assimilation was associated to a lower stomatal conductance and a lower transpiration rate in the absence of tilling. These results are in agreement with those previously obtained in winter wheat by Hou et al. (2013). These authors showed that both the rate of photosynthesis and WUEint increased under rotational tilling conditions, thus enhancing water conservation capacity and improving crop photosynthetic characteristics. In addition, we showed that the presence of cc was another key determinant for improving PWUE under both NT and CT.

Increased PWUE and A_{sat} under NT conditions, especially in the presence of cc, appear to be largely dependent upon the amount of water stored in the soil. Therefore, logically, tillage had a negative impact on SWC even in the presence of cc under both low and optimal fertilization conditions. This result can be explained by the fact that the absence of tilling reduces water loss from the soil by evaporation (Chen et al. 2013), while favoring the development of a deep root system that will capture water more efficiently, notably in the upper layers of the soil (Merrill et al.1996). Moreover, several previous studies have shown that water deficit produces negative effects on A_{sat} (Kumar et al. 2011) due to stomatal closure, which limits the entry of CO_2 into the leaf (Grassi and Magnani 2005; Ripley et al. 2007).

As was similar to that with PWUE, PNUE was also higher under NT conditions even in the absence of N fertilization. This result was due to an increase in A_{sat} and SLA which are two leaf physiological parameters known to be strongly correlated with PNUE (Poorter and Evans 1998; Schulze 2005).

Additionally, we observed that both PWUE and PNUE were higher under optimal N fertilization conditions than under the other conditions. This result was due to an increase in the rate of photosynthesis rather than an increase in the leaf N concentration. Such an increase occurred simultaneously with the increase in the transpiration rate and the stomatal conductance in response to N application. It is likely that N fertilization accelerates the transport of photosynthetic CO_2 in the leaves, leading to enhanced A_{sat} of the plant (Cabrera-Bosquet et al. 2007; Shangguan et al. 2000). The finding that there is a good and positive correlation between A_{sat}, SLA, LAR, and CCI markers and an increase in photosynthetic capacity of the leaf WUE and PNUE is in line with this observation.

In previous studies, a negative correlation between PWUE and PNUE has been documented (Cabrera-Bosquet et al. 2007; Livingston et al. 1999; Maranville and Madhavan 2002). As shown in Fig. 4, we observed that the highest correlations were obtained between traits related to the leaf photosynthetic activity ($A_{sat,}$ $g_{s,}$ and E) and N- and water-use efficiency. Thus, our results showed that a high leaf water-use efficiency is not necessarily correlated with a low PNUE.

PCA analysis allowed the correlations observed between traits related to the leaf photosynthetic efficiency (WUEins, WUEint, PNUE), SWC, the corresponding phenotypic and physiological traits (SLA, LAR, Nmass, A_{sat}, g_s, E, CCI) and their relationship with the tillage system according to the level of N fertilization to be refined. Irrespective of the tillage and of the cover cropping system, the plots that did not receive additional N fertilization (N0) were clearly separated from those fertilized with mineral N (NX). This result shows that the level of N fertilization had a marked impact on the different PWUE and PNUE traits, notably those related to leaf photosynthetic efficiency. Moreover, we observed that in the NX plots, those with cc that were not been tilled were grouped in a separate cluster. These NTcc plot conditions were characterized by lower g_s and E, and by higher WUEint, WUEins, PNUE, LAR, and A_{sat}. This analysis thus confirmed that the no-till system with cover crops had a positive impact on both leaf WUE and PNUE when N fertilization was optimal.

In conclusion, our results indicate that both physiological WUE and PNUE improvement can be achieved by combining agricultural management practices such as no-till and cover cropping notably under high N fertilization inputs.

Competing interests

The authors declare that they have no competing interests.

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