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Title page

# Long-term effects of no tillage treatment on soil N availability, N uptake, and $^{15}$N-fertilizer recovery of durum wheat differ in relation to crop sequence

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# Long-term effects of no tillage treatment on soil N availability, N uptake, and $^{15}$N-fertilizer recovery of durum wheat differ in relation to crop sequence

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## Abstract

No tillage (NT) soil management has largely been promoted because of its potential to generate both economic and environmental benefits. However, it often leads to reductions in crop yield and quality, which in many cases have been attributed to the effects this technique has on the nitrogen (N) dynamics in the soil–plant system. This 2-year study, performed within a long-term experiment in which NT was continuously applied for over 15 years, aimed to verify whether and to what extent the use of NT affects soil N availability, recovery of $^{15}$N-labeled fertilizer, and N use efficiency (NUE) and its components (N uptake efficiency, NUpE; N utilization efficiency, NUtE). Durum wheat was the focal crop. NT was evaluated and compared with conventional tillage (CT) within three crop sequences: continuous wheat (WW), wheat–faba bean (WF), and wheat–berseem clover (WB). At the same time, the timing of N fertilization was varied (either distributing 100 kg N ha$^{-1}$ all at once at crop emergence or applying 50% at crop emergence and 50% at the end of tillering; no N-fertilizer treatment was included as a control). The data indicated that, compared to CT, NT had a detrimental effect on wheat productivity in WW but improved yields in WF and WB. NT was associated with less N uptake by wheat, mainly attributable to a decrease in soil N availability, and to a lesser extent, to a decrease in the $^{15}$N-fertilizer recovery. This reduction in uptake was markedly more evident in WW than in WF or WB, and when all of the $^{15}$N-fertilizer was applied at crop emergence. The effects of tillage system on NUE varied by crop sequence: NT increased NUE (+18% on average compared to CT) in WF, but had the opposite effect in WW (–17% on average). These results suggest that the adoption of the NT technique by farmers must be accompanied by a reorganization of the components of crop management, such as crop rotation and the rate and timing of N fertilization.

## Keywords

No tillage, conventional tillage, NUE, NUpE, NUtE, $^{15}$N-fertilizer recovery, Mediterranean environment

**Abbreviations:** NT, no tillage; CT, conventional tillage; NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; %$^{15}N_{REC}$, percentage of $^{15}$N-fertilizer recovery; WW, continuous wheat; WF, wheat–faba bean; WB, wheat–berseem clover.

## 1. Introduction

No tillage (NT) is defined as "a conservation farming system, in which seeds are placed into otherwise untilled soil by opening a narrow slot, trench, or hole of only sufficient width and depth to obtain proper seed placement and coverage" (Derpsch et al., 2014). It is considered by many to be an environmentally friendly soil management technique that can help enable sustainable development due to its potential to generate economic, environmental, and social benefits. Such benefits, tested against the conventional tillage (CT) technique (usually based on moldboard plowing), include mitigation of soil erosion (Scopel et al., 2005), enhanced aggregation and aggregate stability (Madari et al., 2005), reduced fuel consumption (up to 70% in fuel savings have been reported; FAO, 2008), and savings in labor and time (Kirkegaard, 2010). Moreover, NT may lead to greater soil carbon sequestration (González-Sánchez et al., 2012) and thus help reduce global warming, although increases in nitrous oxide emissions following the adoption of NT could offset this effect (Baggs et al., 2003). Furthermore, often NT tends to conserve soil water better than CT, and this effect is particularly evident during dry periods (Lampurlanés et al., 2002; Amato et al., 2013; Ruisi et al., 2014). This often has positive effects on crop growth and yield (De Vita et al., 2007; Cullum, 2012; Giambalvo et al., 2012; Ruisi et al., 2012), but contradictory yield results have been reported in comparisons of NT and CT (Hernanz et al., 2002; Mazzoncini et al., 2008).

In many cases, reductions in yield due to the adoption of NT are attributable to the effects that this technique has on the nitrogen (N) dynamics in the soil–plant system (Lundy et al., 2015; Pittelkow et al., 2015). In fact, a tillage system may affect the fate of N via changes in the soil structure, placement of crop residues, organic matter decomposition, and water availability for the crop (Karlen et al., 1998). Soil cultivation that improves soil aeration and mixing and incorporates crop residues into the soil generally stimulates the decomposition of organic matter, leading to a faster release of N from residue compared to surface-placed residues in NT systems (Varco et al., 1993; Watson et al., 2002). On the other hand, retaining crop residues on the soil surface when using NT can increase the immobilization rate of both indigenous soil N and fertilizer N (Rice and Smith, 1984; Dawson et al., 2008). It can also sometimes lead to N losses from soil through volatilization, denitrification, and leaching (Velthof et al., 2002; de Ruijter et al., 2010; Agneessens et al., 2014). Thus, higher soil N availability is often reported under CT than NT (Six et al., 2004; Peigné et al., 2007). Moreover, the application of NT compared to CT may lead to a reduction in N-fertilizer recovery, although the differences between the two tillage techniques can vary widely in relation to the method of N-fertilizer placement (e.g., broadcast, sidebanding, below seed row), N sources, and N fertilization timing (Malhi et al., 2001). Conversely, under reduced tillage or NT, N mineralization rates can be increased compared to plowed systems as a result of an increase in the amount of both soil organic carbon and soil organic N pools, and an intensification of soil microbial activity (Sharifi et al., 2008). Moreover, surface crop residues help to conserve soil moisture and mitigate extreme soil temperatures, thereby generating favorable conditions for soil microbial activity during dry years and consequently increasing soil N mineralization (van Donk et al., 2010; Moreno-Cornejo et al., 2014). Obviously, many factors can be responsible for discrepancies in soil N availability under CT and NT, including climatic conditions, soil characteristics, management practices, and the duration of experiments. For example, some studies have reported that the effects of tillage system on N cycling can vary markedly according to crop sequence due to a synergistic effect of these two factors on soil organic matter, water retention, and microbial activity (López-Bellido et al., 1997; Lafond et al., 2006; Pala et al., 2007; Melero et al., 2011; Amato et al., 2013).

To shed light on the discussion regarding the effects of tillage system on N dynamics, N availability, and N-fertilizer recovery, and considering the limited number of studies conducted in Mediterranean areas on this topic, we performed a 2-year study to verify whether, and if so to what extent, the continuous application of NT affects soil N availability, recovery of $^{15}$N-labeled fertilizer, and N use efficiency (NUE) and its components (N uptake efficiency, NUpE; N utilization efficiency, NUtE). We used durum wheat as the focal crop, and conducted the study within a long-term experiment during which NT was continuously applied for over 15 years. We also varied both the crop sequence and timing of N fertilization. Our results will be useful for helping farmers to plan correct N fertilization strategies to increase the sustainability of cropping systems by improving the use efficiency of both native and auxiliary resources.

## 2. Materials and methods

### *2.1. Site characteristics*

A long-term field experiment was started in the 1991–1992 growing season at Pietranera farm, which is located about 30 km north of Agrigento, Sicily, Italy (37°30’N, 13°31’E; 178 m a.s.l.), on a deep, well-structured soil classified as a Chromic Haploxerert (Vertisol). Soil characteristics (measured at the beginning of the experiment and referring to the 0–0.40 m layer) were as follows: 52.5% clay, 21.6% silt, 25.9% sand, pH 8.1 (1:2.5 $H_2O$), 1.40% total C (Walkley Black), 1.29 g kg$^{-1}$ total N (Kjeldahl), 36 mg kg$^{-1}$ available P (Olsen), 340 mg kg$^{-1}$ $K_2O$ (exchangeable potassium), cation exchange capacity 35 $\text{cmol}_+$ $\text{kg}^{-1}$, 0.38 $\text{cm}^3$ $\text{cm}^{-3}$ water content at field capacity (pF 2.5), and 0.16 $\text{cm}^3$ $\text{cm}^{-3}$ permanent wilting point (pF 4.5). The climate of the experimental site is semiarid Mediterranean, with a mean annual rainfall of 581 mm, mostly in autumn-winter (74%) and in spring (18%). The dry period is from May to September. The mean air temperature is 15.9°C in autumn, 9.8°C in winter, and 16.5°C in spring. The average minimum and maximum annual temperatures are 10.0°C and 23.3°C, respectively. The weather data were collected from a weather station located within 500 m of the site.

### *2.2. Experimental design and crop management*

The experiment was set up as a strip-plot design with two replications. Three soil tillage systems (CT, NT, and reduced tillage [RT]) served as vertical treatments and three crop sequences (continuous wheat, WW; wheat–faba bean, WF; and wheat–berseem clover, WB) served as horizontal treatments. CT consisted of one moldboard plowing to a depth of 0.30 m in the summer, followed by one or two shallow hallowing operations before planting. In the RT plots, primary tillage consisted of chisel plowing to a depth of 0.40 m (non-inverting action) in the summer followed by moldboard plowing to a depth of 0.15 m (after the first rains of autumn) and followed by one shallow harrowing operation to prepare a proper seedbed: the moldboard plowing operation was omitted beginning in the eighth year of the experiment (1998–1999). Finally, NT consisted of sowing by direct drilling. Wheat was planted in rows spaced 0.16 m apart (for all tillage treatments), always at 350 viable seeds $\text{m}^{-2}$, while faba bean and berseem clover were sown at 40 and 1200 viable seeds $\text{m}^{-2}$, respectively, with an inter-row spacing of 0.75 m for faba bean and 0.16 m for berseem clover. Cultivation and tillage treatments were performed with commercial farm equipment. The plot size was 370 $\text{m}^2$ (18.5 × 20.0 m). Each year, both rotations (WF and WB) were duplicated in reverse order so as to obtain data annually from all crops. In NT plots, weeds were controlled with glyphosate (N-[phosphonomethyl] glycine) before planting at a dose of 533–1066 g a.e. $\text{ha}^{-1}$, depending on the development of weeds. During the wheat growing season, weeds were controlled by applying post-emergence herbicides at the early growth stage of the crop, with no differences among the three tillage systems. In the faba bean plots, weeds were controlled mechanically by shallow hoeing. Berseem clover and faba bean were harvested in June or July each year, leaving standing straw; loose residues were spread uniformly throughout the plot. Wheat stubble (about 20–25 cm from the soil surface) was left standing and the straw was baled and removed from the field. The soil surface was covered by mulch in the NT treatments, and the coverage was always >30%. More details on how the trial was performed are reported in Giambalvo et al. (2012) and Amato et al. (2013).

Within the framework of this experiment, after 15 years of continuous application of the treatments, we conducted a 2-year (2005–2006 and 2007–2008) in-depth study to evaluate how N uptake, NUE (and its components), and $^{15}$N-fertilizer recovery of durum wheat vary when tillage system, crop sequence, and N fertilizer timing vary. This study considered only NT and CT plots. Each year, all plots to be allocated to wheat were divided into three subplots (18.5 × 6.6 m), in which N fertilizer was applied as follows: $N_0$, no N fertilizer; $N_{100}$, 100 kg N ha$^{-1}$ all at once, distributed at crop emergence; and $N_{50\text{-}50}$, 100 kg N ha$^{-1}$ split into two treatments, 50% at crop emergence and 50% at the end of tillering.

Within each subplot, four microplots (2 × 2 m) were identified at the emergence of durum wheat. All microplots within the $N_{100}$ and $N_{50\text{-}50}$ subplots were labeled with $^{15}$N fertilizer [100 kg N ha$^{-1}$ as $(NH_4)_2SO_4$ with an isotopic enrichment of 1.33 atom%] added to the area as scheduled (i.e., all at crop emergence or split), following the application procedure described by Høgh-Jensen and Schjoerring (1994); the rest of the subplots outside of the $^{15}$N-labeled areas received equivalent amounts of unlabeled fertilizer.

Soil samples (0–0.40 m layer) were collected from each subplot immediately before sowing and soon after wheat harvest and analyzed for 2M KCl-extractable $NH_4$–N and $NO_3$–N using a Bran & Luebbe II AutoAnalyzer.

At harvest, a sample of total aboveground plant material from the center of each microplot was taken, oven-dried at 60ºC for 72 h, weighed, ground to a fine powder (sieved using a 0.1 mm mesh) in a fast running mill, and analyzed for total N and $^{15}$N enrichment. The concentrations of total N and $^{15}$N were determined through elemental analyzer-isotope ratio mass spectrometry (Carlo Erba NA 1500). Plant height, biomass production, grain yield and its components (spike number per square meter, kernel number per spike, 1000-kernel weight), and N grain content were recorded.

### *2.3. Calculations and statistical analyses*

Data on $^{15}$N enrichment of biomass were used to calculate the labeled-fertilizer N recovery ($^{15}N_{REC}$) on an area basis (kg N ha$^{-1}$) and percentage basis, according to Hauck and Bremner (1976):

$$^{15}N_{REC} = N_t \times \frac{^{15}N_{fp} - {}^{15}N_{nfp}}{^{15}N_{fert} - {}^{15}N_{nfp}}$$

and

$$\%^{15}N_{REC} = \frac{^{15}N_{REC}}{f} \times 100$$

where $N_t$ was the plant N content measured at maturity (kg ha$^{-1}$), $^{15}N_{fp}$ was the atom% $^{15}N$ in the fertilized plants, $^{15}N_{nfp}$ was the atom% $^{15}N$ in the non-fertilized plants, $^{15}N_{fert}$ was the atom% $^{15}N$ in the fertilizer, and $f$ was the fertilizer rate (kg N ha$^{-1}$).

Nitrogen efficiency parameters were calculated according to Moll et al. (1982) and Huggins and Pan (1993). NUE was defined as the ratio of grain produced (Gw, kg ha$^{-1}$) to N supply (i.e., the soil N potentially available for the crop; Ns, kg N ha$^{-1}$), where Ns was estimated as the amount of applied N (f) plus $N_t$ plus residual post-harvest N in the soil (Nsph, kg N ha$^{-1}$), both determined from control subplots (no applied N). NUpE was calculated as Nt/Ns. NUtE was determined as Gw/$N_t$. Nitrogen harvest index (NHI; %*)* was calculated as the ratio of N in grain to $N_t$.

For all measured variables, normality was tested using the Shapiro–Wilk test of normality. All variables corresponding to proportions were arcsine transformed before analysis to ensure a better fit with the Gaussian law distribution. Data from each year were analyzed separately, and homogeneity of variances was assessed using Bartlett’s test before combined analyses were performed. Data were analyzed according to a strip-split-plot design with crop sequence, tillage system, and N fertilization as fixed factors, and year and replicates as random factors. Treatment means were compared using Fisher’s protected least significant difference (LSD) test at the 5% probability level.

## 3. Results

### *3.1. Weather conditions*

The weather conditions during the experimental period are shown in Figure 1. Total rainfall was 508 mm in 2005–2006, 12% lower than the long-term average for the area, and 443 mm in 2007–2008, 24% lower than the long-term average. The mean monthly temperature was 16.0°C in 2005–2006 and 16.9°C in 2007–2008, very similar to the long-term mean temperature (16.6°C).

### *3.2. Biomass and grain yield, N uptake, and grain protein content*

Table 1 presents the results of the statistical analyses for the effects of the applied treatments and their interactions on wheat yield and yield components, and N efficiency parameters. On average, biomass and grain yield of durum wheat were markedly lower in WW than in WB and WF (Fig. 2A,B). The effects of tillage system on biomass and grain yield greatly varied by crop sequence (the tillage × crop sequence interaction was significant at $P<0.001$). In WW, NT dramatically reduced biomass and grain yield compared to CT (–36% and –37%, respectively). In WB and WF, grain yield was 5% and 9% higher, respectively, under NT than CT but no differences were observed for biomass between the two tillage systems. The effects of tillage system and crop sequence on the number of spikes per square meter and the number of kernels per spike were similar to those observed for biomass (Fig. 3A,B). On the contrary, NT increased 1000-kernel weight compared to CT in WB and WF but not in WW (Fig. 3C). NT decreased total N uptake compared to CT in all crop sequences, and this reduction was stronger in WW (–38%) than WB and WF (–8% and –13%, respectively; Fig. 4A). Grain protein content was lower in NT than CT in all crop sequences with a stronger decrease in WF (–15%) than WB and WW (–6% and –3%, respectively; Fig. 4B).

On average, N fertilization (irrespective of fertilizer timing) increased total N uptake (+38% on average) and grain protein content (+16% on average). Regarding both biomass and grain yield, the effects of N fertilization varied by crop sequence (the N fertilization × crop sequence interaction was significant at $P<0.01$; Fig. 5A,B); in particular, fertilization with 100 kg N ha$^{-1}$ (irrespective of fertilizer timing) resulted in an average increase in biomass and grain yield only in WW, not in either legume treatment. For the other traits, the interactions between N fertilization and the other treatments were never significant.

### *3.3. N efficiency and recovery of $^{15}$N-fertilizer*

On average, the amount of soil N potentially available for the crop (i.e., the N supply), the amount of N derived from soil (Ndfs), and the recovery of $^{15}$N-fertilizer ($^{15}N_{REC}$) were all higher when wheat was grown after berseem clover or faba bean than in continuous wheat. On the whole, these traits were higher under CT than NT, and the differences between the two tillage techniques differed among crop sequences, being higher in WW than WF and WB (Fig. 6 A–C).

The effects of tillage system on NUE varied by crop sequence (the tillage × crop sequence interaction was significant at $P<0.001$; Table 1 and Fig. 7A). In particular, compared to CT, NT increased NUE from 19.2 to 22.7 in WF but had the opposite effect in WW, decreasing it from 23.4 to 19.4; no differences due to the tillage system were observed in WB. On average, NT led to a reduction of NUpE compared to CT but the detrimental effect varied by crop sequence, being greater in WW than in WB and WF (Fig. 7B). The application of NT instead of CT led to an increase in NUtE only in WB and in WF, whereas no significant effect was observed in WW (Fig. 7C). The NHI was higher in NT than CT in WB and WF but not in WW (data not shown).

On average, N fertilization (irrespective of fertilizer timing) resulted in a 35% reduction of NUE compared to the $N_0$ treatment, due to a reduction of both NUpE (–16%, on average) and NUtE (–22%, on average). No differences were observed in the other three indices due to the N fertilizer timing. Splitting the fertilizer ($N_{50\text{-}50}$) increased $^{15}N_{REC}$ compared to the $N_{100}$ treatment in NT but not in CT (Fig. 8).

## 4. Discussion

The results of our research suggest that the NT technique may be a valuable alternative to CT in the Mediterranean environment, as it leads to equivalent or even higher yields compared to CT, as long as it is accompanied by a rational crop sequence. In fact, in our experiment, NT had a detrimental effect on wheat productivity (compared to CT) in the continuous wheat system but improved the yield of wheat grown after a legume crop (both faba bean and berseem clover). Our results are in agreement with previous studies (Rusinamhodzi et. al., 2011; Pittelkow et al., 2015) that showed that crop rotation, when targeted appropriately, can help minimize the negative impact of NT on crop productivity. In fact, the adoption of NT instead of CT in monocultural systems (WW in our case) may amplify problems related to soil-borne pathogens, and at the same time, may cause a reduction in soil nutrients (mainly N) available for the crop (Hernanz et al., 2002; Rusinamhodzi et. al., 2011).

In the present research, N uptake was, on average, lower with NT than with CT; this result is in agreement with the findings of other authors (Ishaq et al., 2001; Soon et al., 2006). Our data seem to suggest that this was mainly attributable to a reduction of soil N available to plants under NT compared to CT, and only in small part to a lower efficiency in N uptake (i.e., in the NUpE). According to Silgram and Shepherd (1999), soil cultivation can enhance the decomposition of organic matter by altering the structure, temperature, and aeration of soil, as well as the distribution of crop residues along the soil profile and the degree of physical protection of organic matter from microorganisms or their enzymes. Watson et al. (2002) found that tillage stimulates microbial activity in the soil, which increases N availability. Other authors have emphasized that a key factor reducing soil N under NT conditions compared to CT is the presence of crop residues on the soil surface, which can increase the N immobilization rate, thus decreasing its availability to the crop (Erenstein, 2002; Dawson et al., 2008; Giller et al., 2009). In this study, the differences found in the available soil N between CT and NT were marked in the continuous wheat plot (–30% in NT compared to CT) and, on the whole, they were small when wheat was grown in rotation with a legume crop (–7% on average). It is possible that the retention of cereal crop residues (which have a high C:N ratio, unlike legume crops) on the soil surface may have exacerbated the N stress in WW by temporary N immobilization (as already highlighted by Erenstein 2002), and as a consequence, led to a lower N availability for the crop.

Even though organic N mineralization rates are often higher in plowed systems, a gradual accumulation of greater amounts of organic matter in NT systems over time may compensate for this effect (Salinas-Garcia et al., 1997). Rice et al. (1986) suggested that the lower availability of N frequently observed in NT soils can in some cases be a transient effect; in their experiment, availability of soil N in NT apparently approached that of CT after approximately 10 years. However, our data on soil N availability, which were collected after 14–16 years of continuous application of CT and NT, do not allow us to hypothesize on the occurrence of a transient effect.

In our experiment, N fertilization (irrespective of fertilizer timing) resulted in increases in wheat grain yield in WW but not in WB and WF. Once again, this result can be attributable to different soil N availability among the three crop sequences; in fact, soil N availability was markedly lower in WW than WB and WF, and it is well known that crop responses to N fertilization are greater under conditions of low soil N availability (Godard et al., 2008). The increase in soil N availability (calculated in $N_0$ plots on the basis of N uptake by wheat and the amount of residual N in soil at harvest) in WB and WF compared to WW was on average 60–70 kg N $ha^{-1}$. This result is not surprising, as other studies (Hauggaard-Nielsen et al., 2009; Giambalvo et al., 2011) have already shown that faba bean and berseem clover are both able to fix high amounts of atmospheric N (even close to 300 kg N $ha^{-1}$), which will then obviously become partly available to the subsequent crop.

We observed a significant decrease in the percentage of $^{15}$N-fertilizer recovery ($\%^{15}N_{REC}$) with the NT technique versus the CT method. This may be attributable to the higher N losses from soil through volatilization under NT compared to CT, due to the lack of incorporation of N fertilizer into the soil (Fox and Piekielek, 1993; Angás et al., 2006). Moreover, the increased potential for N immobilization at the surface of no-till soils may significantly reduce crop recovery of N fertilizer (Rice and Smith, 1984). Considering that this potential for N immobilization varies in relation to the composition of crop residues (proportionally to the C:N ratio), this would also explain why in our experiment the differences in the $\%^{15}N_{REC}$ between CT and NT were higher in WW than in both WB and WF. Moreover, our data indicated improved $\%^{15}N_{REC}$ when N application was split in NT systems but not in CT. This could be explained considering that a greater N immobilization generally occurs under NT with respect to CT during the early growth stages of a crop, as previously observed by other authors (Haugen Kozyra et al., 1993; Melaj et al., 2003). Therefore, splitting N fertilization into two fractions (i.e., in our case, applying 50% at crop emergence and 50% at the end of tillering) would reduce the risk of N immobilization under NT conditions, thus increasing the chances for this element to be taken up by the crop. Furthermore, the excess soil water that often accumulates in no-till soils during winter (the rainy season in the Mediterranean environment), when plants are in the early growth stages, along with the lower soil temperatures compared to plowed soils, can slow down initial crop growth, with negative repercussions on N uptake; this, in turn, leads to a greater chance that N will be released into the environment. On the contrary, in soils managed with the CT technique, plants often have a more rapid early growth (which is favored by the higher soil temperatures and the faster drainage compared to no-till soils); this would imply higher N requirements, and therefore, a greater chance that the crop will absorb the N provided by the fertilizer. The higher number of spikes per square meter we observed in CT compared to NT seems to confirm that soil cultivation (i.e., CT) led to more favorable conditions for tillering and plant growth during the vegetative phase of the cycle. The abovementioned negative effects on initial crop growth due to NT application are generally widely counteracted by the greater availability of water for the crop during the spring, attributable to reduced soil water evaporation with NT (Blevins and Frye, 1993; Lampurlanés and Cantero-Martínez, 2006).

A meta-analysis by Lundy et al. (2015) evaluated the influences of crop and environmental variables on no-till productivity, and found that N fertilization reduces yield declines following no-till adoption and that this effect is more marked in tropical/subtropical regions than in temperate regions. Our results disagree with their findings, as the interaction of tillage system × N fertilization was not significant for grain yield. On the other hand, we did not observe a yield decline following NT adoption when a legume crop was included in the crop sequence; that is, a marked yield decline due to the adoption of NT was observed only in the continuous wheat plot. Therefore, our data suggest that the yield decline related to the adoption of NT in WW must be attributed to factors beyond N deficiency, which could be overcome by increasing N-fertilizer rate (as suggested by Alvarez and Steinbach, 2009);such factors may include an increased occurrence of crop diseases, increased weed competition, and so forth. This seems more plausible when we consider that the wheat monoculture included in this study has been practiced continuously for over 15 years.

Considering the lower N availability associated with NT compared to CT systems as well as the lower efficiency of N-fertilizer utilization observed under NT soil, it is not surprising that, on average, a lower grain protein content was found with NT than with CT. This result agrees with the findings of López-Bellido et al. (1998) and McConkey et al. (2002). Finally, we found that the lower grain protein content obtained with NT was particularly accentuated when wheat was grown after a legume crop (in both WF and WB plots), whereas the differences between NT and CT were smaller in WW. This result may be associated with the effects of tillage on wheat grain yield and N uptake, which both varied by crop sequence.

Tillage system affected the N efficiency indices (NUE, NUpE, and NUtE) differently according to crop sequence. Under WW, the application of NT led to a decrease in NUE attributable to a reduction in grain yield from CT to NT that was much higher than that observed for N supply. This suggests that, in WW, reductions in grain yield observed in NT compared to CT were due not only to changes in N supply but also to other factors. In this regard, an increase in the incidence of some pathogens of wheat (*Gaeumannomyces graminis*, *Rhizoctonia solani*, *Pythium* spp., and other species belonging to the genus *Fusarium*) that we noticed in plants grown under NT compared to CT may have played an important role in the WW system. On the other hand, it has already been shown that when NT and continuous wheat (retaining wheat residues on the soil surface) are combined, some residue-borne pathogens can progressively become extremely problematic (Bockus and Shroyer, 1998; Paulitz et al., 2002). This hypothesis would also explain the reduction of NuPE found in WW (but not in WB and WF) due to the application of NT.

N fertilization (irrespective of timing) resulted in a reduction of NUE and both of its components, NUpE and NUtE, in agreement with other studies on wheat (Sylvester-Bradley and Kindred 2009, Giambalvo et al. 2010, López-Bellido and López-Bellido 2001), without interacting with tillage or crop sequence.

## 5. Conclusions

The results of the present experiment, performed within a long-term field experiment on a Vertisol under rainfed Mediterranean conditions, show that the NT technique is a valid alternative to CT (based on moldboard plowing) as it can ensure equivalent or even higher yields, as long as it is accompanied by a rational crop sequence. Moreover, our findings highlight that the application of NT, compared to CT, leads to reductions in both native soil mineral N (due to a reduced organic matter mineralization rate, increased mineral N immobilization rate, and increased N losses) and N-fertilizer recovery (%$^{15}$N$_{REC}$). For the latter trait, the observed reductions following the application of NT were markedly more evident in WW than in WF or WB, and when the N-fertilizer was applied all at the same time, at crop emergence (N$_{100}$). This suggests that the adoption of the NT technique by farmers, which in our opinion is desirable considering the numerous agronomic and environmental benefits that this technique can generate, must be accompanied by a reorganization of the components of crop management, such as crop rotation and the rate and timing of N fertilization.

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## Figure legends

Fig. 1. Accumulated rainfall (A) and 10-day mean air temperature (B) at the experimental site during the two growing seasons (2005–2006 and 2007–2008); 30-year average 10-day temperatures and accumulated rainfall are also included.

Fig. 2. Biomass [A] and grain [B] yields of wheat grown under conventional tillage (CT) and no tillage (NT) approaches in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous wheat). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.

Fig. 3. Grain yield components (number of spikes per square meter [A]; number of kernels per spike [B]; 1000-kernel weight [C]) of wheat grown under conventional tillage (CT) and no tillage (NT) in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous wheat). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.

Fig. 4. Nitrogen uptake [A] and grain protein content [B] of wheat grown under conventional tillage (CT) and no tillage (NT) in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous wheat). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.

Fig. 5. Biomass [A] and grain [B] yields of wheat as affected by N fertilization (no N fertilizer, $N_0$; 100 kg N ha$^{-1}$ all distributed at crop emergence, $N_{100}$; 100 kg N ha$^{-1}$ split distribution, using 50% at crop emergence and 50% at the end of tillering, $N_{50\text{-}50}$) in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous wheat). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.

Fig. 6. Amount of potentially available N in soil for crops (N supply [A], determined on sub-plots with no N applied), amount of N derived from soil (Ndfs [B]), and recovery of $^{15}$N-fertilizer ($^{15}N_{REC}$ [C]) in wheat grown under conventional tillage (CT) and no tillage (NT) in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous wheat). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.

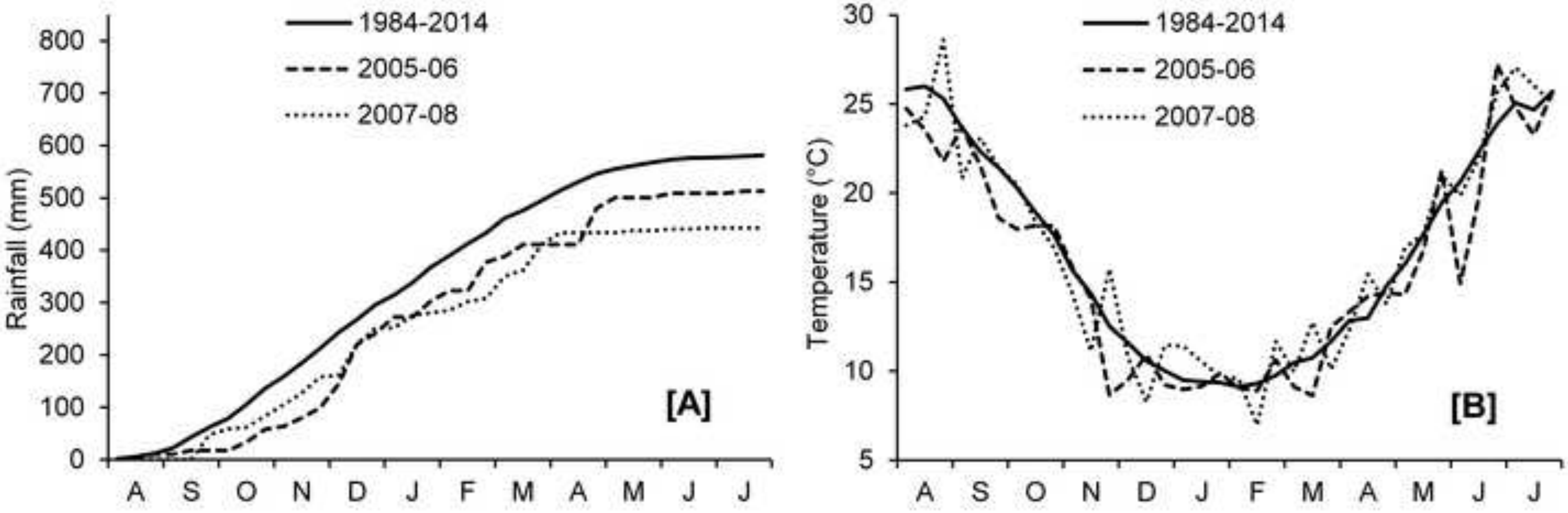
Fig. 7. Nitrogen efficiency indices (N use efficiency, NUE [A]; N uptake efficiency, NUpE [B]; N utilization efficiency, NUtE [C]) in wheat grown under conventional tillage (CT) and no tillage (NT) in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous wheat). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.

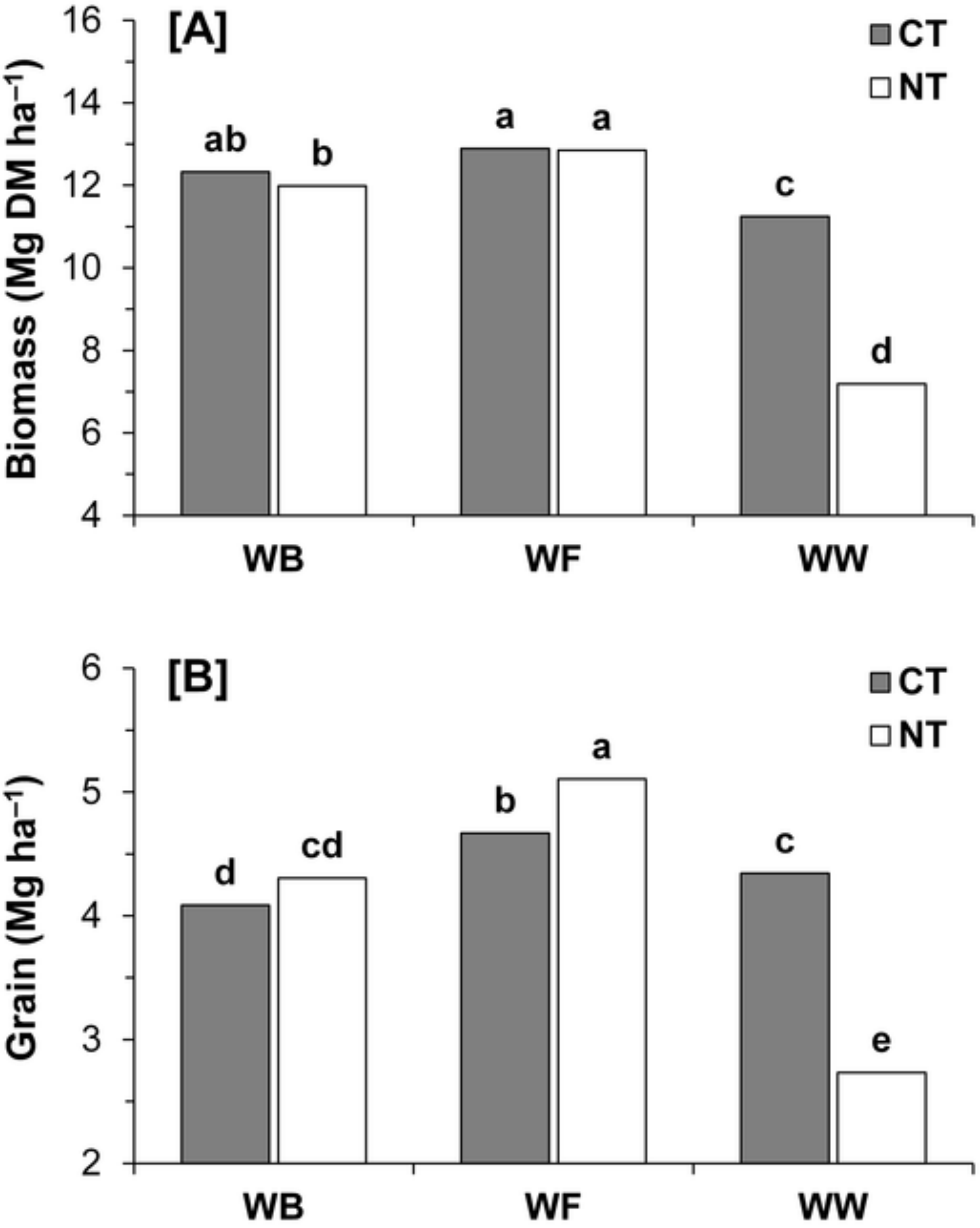
Fig. 8. Recovery of $^{15}$N-fertilizer ($^{15}N_{REC}$) by wheat as affected by tillage system (conventional tillage, CT; no tillage, NT) and N fertilization (100 kg N ha$^{-1}$ all distributed at crop emergence, $N_{100}$; 100 kg N ha$^{-1}$ split distribution, using 50% at crop emergence and 50% at the end of tillering, $N_{50\text{-}50}$). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.

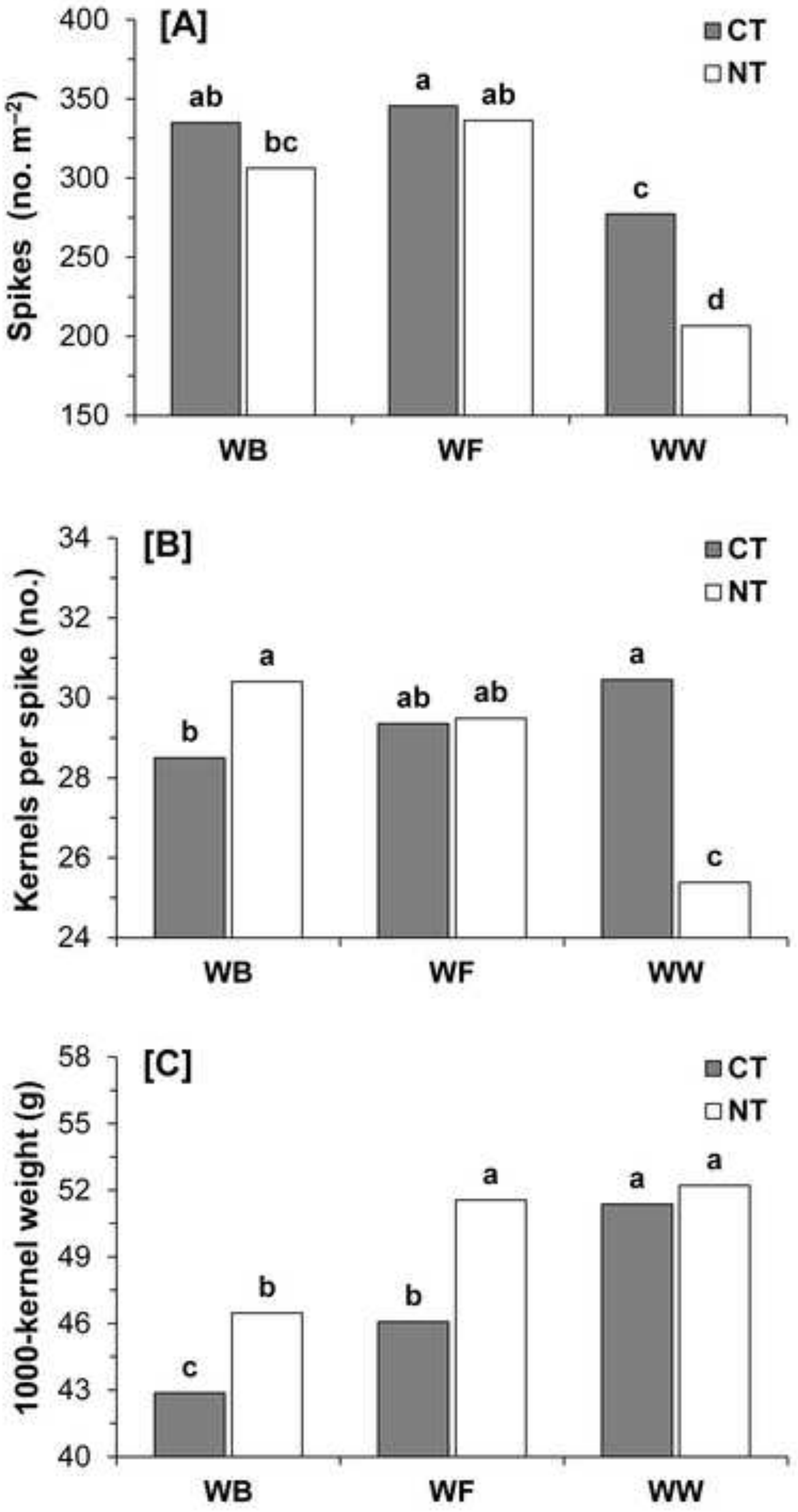
**Table 1**

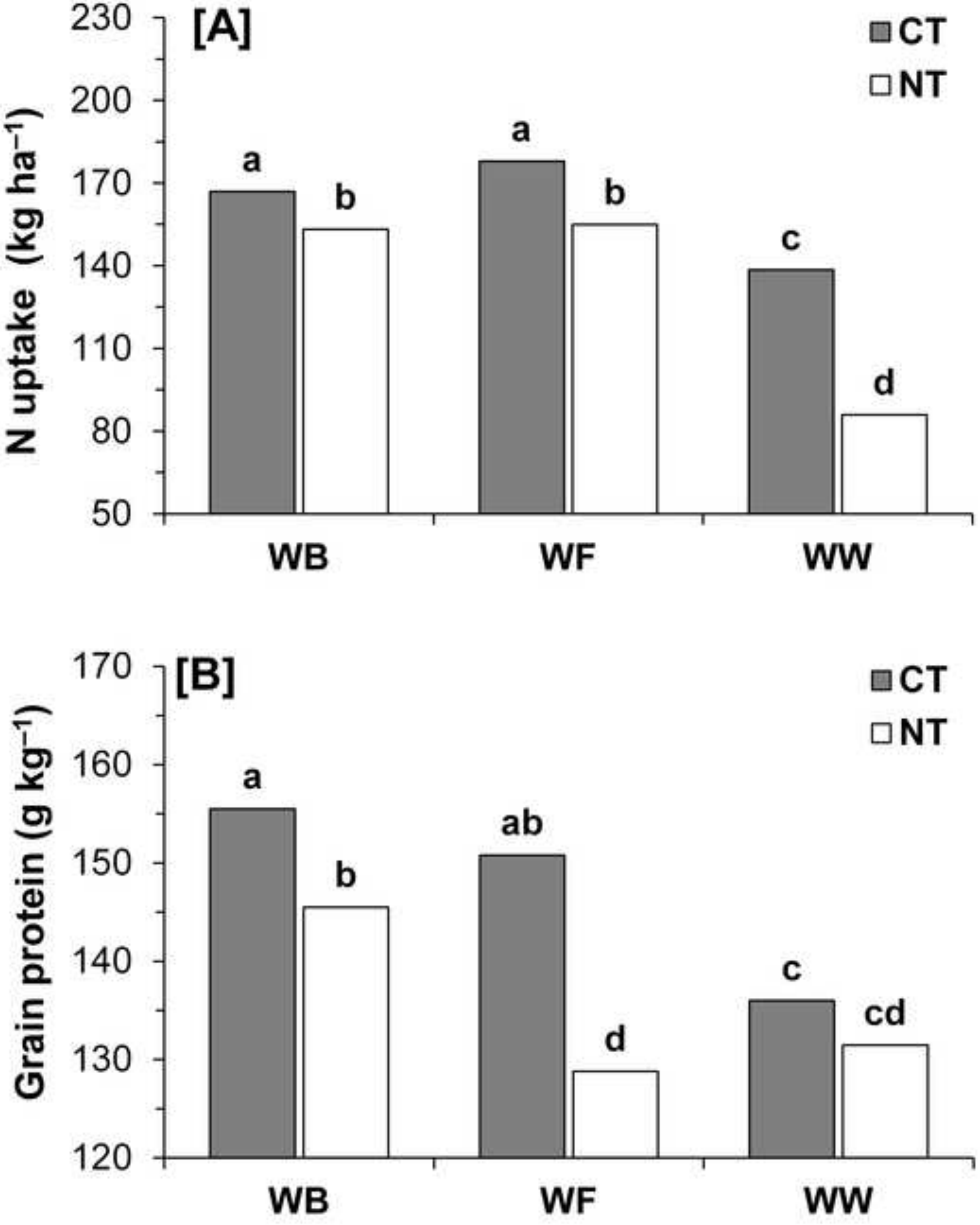
Table 1. *P*-values for the effects of crop sequence, tillage system, and N fertilization on yield and yield components, N uptake, grain protein content, potentially available N in soil for crops (N supply), and N efficiency parameters (Ndfs, nitrogen derived from soil; %$^{15}$N$_{REC}$, labeled-fertilizer nitrogen recovery on a percentage basis; NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency).

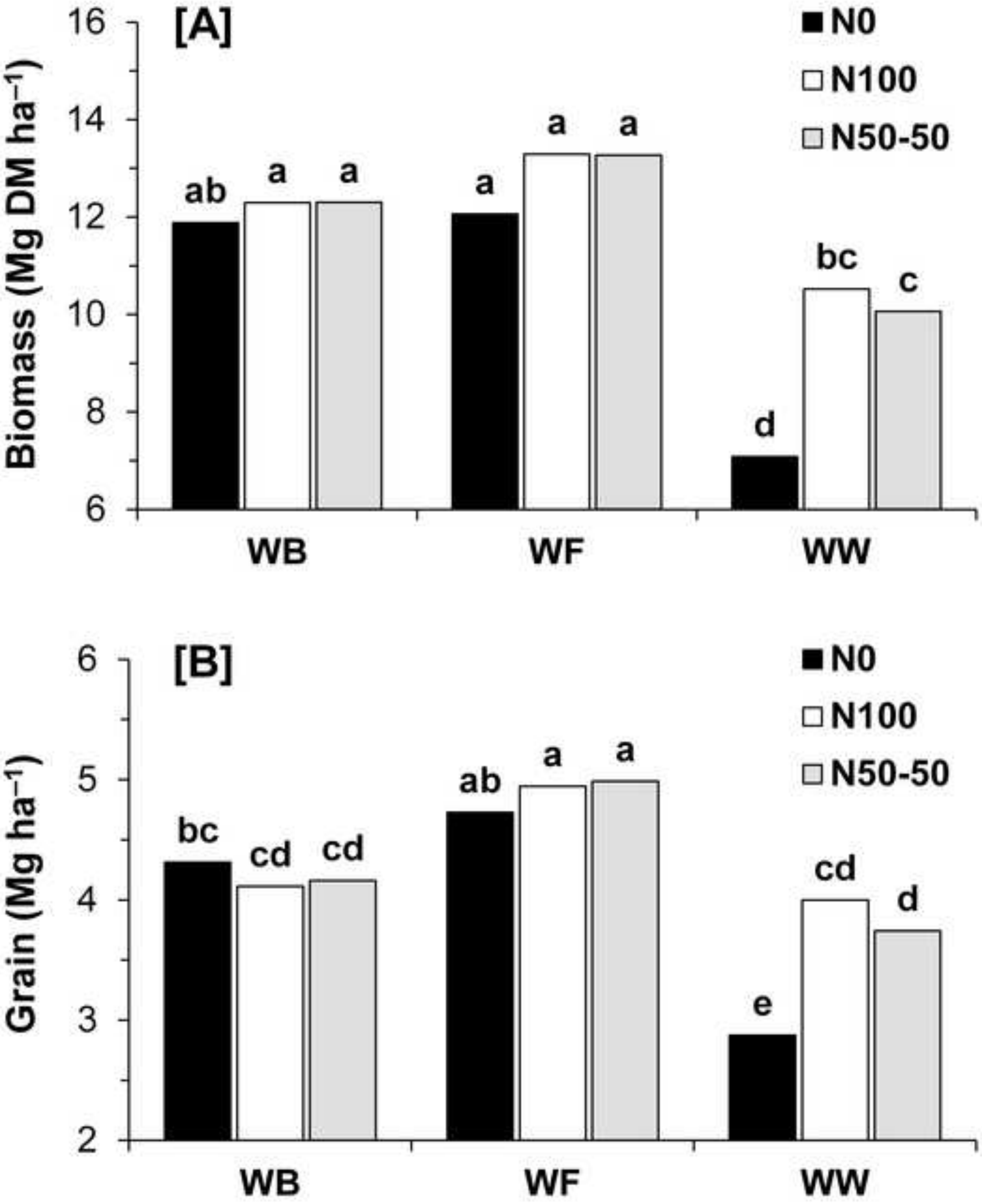
| | Mean effects | | | Interactions | | | |
|---|---|---|---|---|---|---|---|
| | Crop sequence (CS) | Tillage (T) | Fertilization (F) | CS × T | CS × F | T × F | CS × T × F |
| Traits | *P*-value | | | | | | |
| Biomass | <0.001 | <0.001 | <0.001 | <0.001 | 0.049 | ns | ns |
| Grain | <0.001 | <0.001 | ns | <0.001 | <0.001 | ns | ns |
| No. spikes m$^{-2}$ | <0.001 | <0.001 | ns | 0.033 | ns | ns | ns |
| Kernels per spike | 0.007 | ns | ns | <0.001 | ns | ns | ns |
| 1000-kernel weight | <0.001 | 0.002 | 0.022 | 0.001 | ns | ns | ns |
| N uptake | <0.001 | 0.003 | <0.001 | 0.003 | ns | ns | ns |
| Grain protein content | <0.001 | <0.001 | <0.001 | 0.005 | ns | ns | ns |
| N supply | <0.001 | 0.032 | — | 0.044 | — | — | — |
| Ndfs | <0.001 | 0.010 | 0.035 | 0.009 | 0.007 | ns | ns |
| %$^{15}$N$_{REC}$ | <0.001 | 0.013 | 0.012 | 0.046 | ns | 0.049 | ns |
| NUE | 0.003 | ns | <0.001 | <0.001 | ns | ns | ns |
| NUpE | 0.015 | 0.006 | <0.001 | 0.005 | ns | ns | ns |
| NUtE | <0.001 | 0.015 | <0.001 | 0.001 | ns | ns | ns |

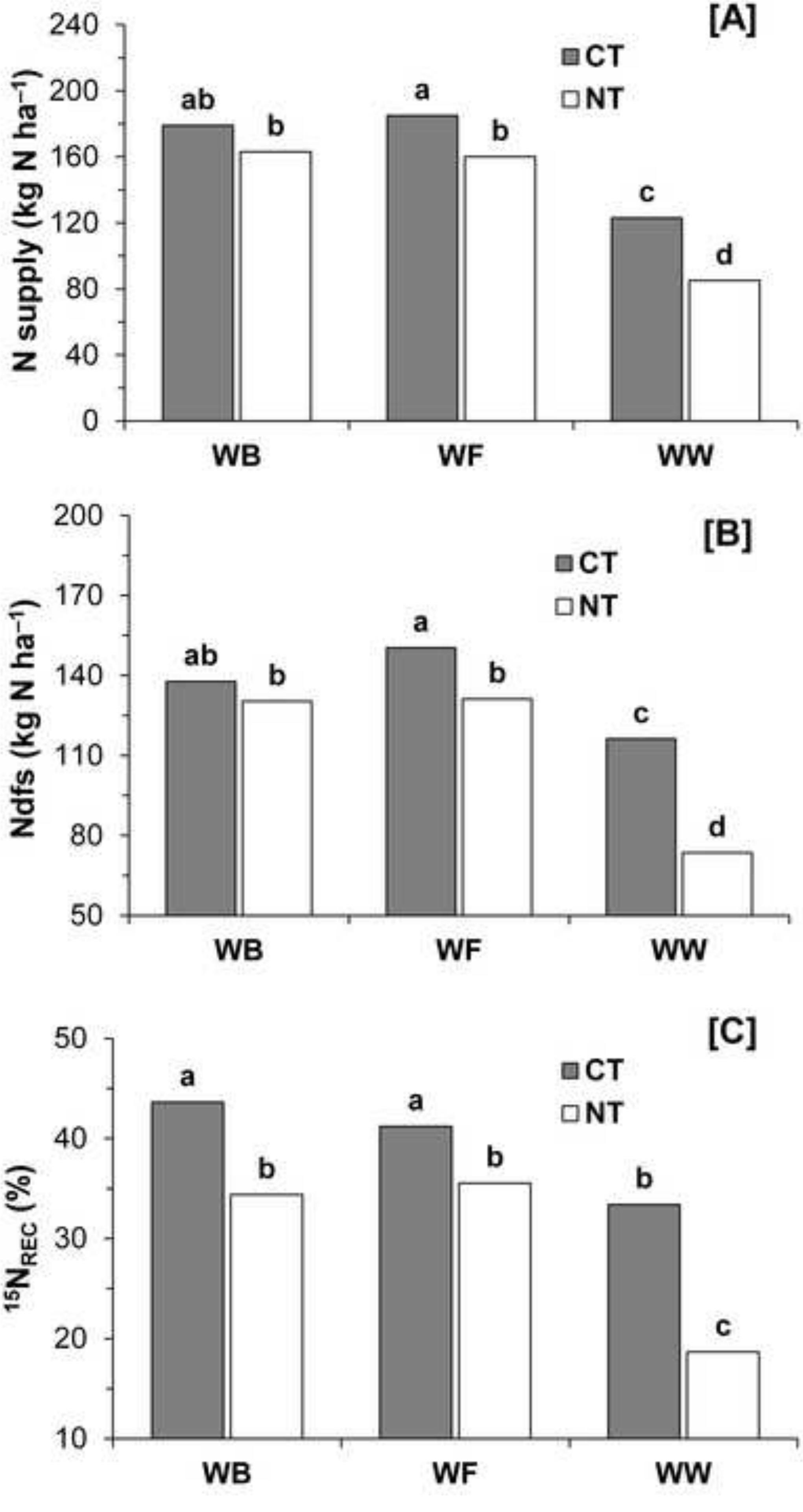

1984-2014
2005-06
2007-08
Rainfall (mm)
0
100
200
300
400
500
600
700
800
A
S
O
N
D
J
F
M
A
M
J
J
[A]
Temperature (°C)
5
10
15
20
25
30
[B]


[A]
■ CT
□ NT
Biomass (Mg DM ha⁻¹)
16
14
12
10
8
6
4
ab
b
a
a
c
d
WB
WF
WW
[B]
■ CT
□ NT
Grain (Mg ha⁻¹)
6
5
4
3
2
d
cd
b
a
c
e
WB
WF
WW


[A]
■ CT
□ NT
Spikes (no. m⁻²)
400
350
300
250
200
150
ab
bc
a
ab
c
d
WB
WF
WW
[B]
■ CT
□ NT
Kernels per spike (no.)
34
32
30
28
26
24
b
a
ab
ab
a
c
WB
WF
WW
[C]
■ CT
□ NT
1000-kernel weight (g)
58
55
52
49
46
43
40
c
b
b
a
a
a
WB
WF
WW


[A]
■ CT
□ NT
N uptake (kg ha⁻¹)
230
200
170
140
110
80
50
a
b
a
b
c
d
WB
WF
WW
[B]
■ CT
□ NT
Grain protein (g kg⁻¹)
170
160
150
140
130
120
a
b
ab
d
c
cd
WB
WF
WW


[A]
Biomass (Mg DM ha⁻¹)
16
14
12
10
8
6
■ N0
□ N100
▨ N50-50
ab
a
a
a
a
a
bc
c
d
WB
WF
WW
[B]
Grain (Mg ha⁻¹)
6
5
4
3
2
■ N0
□ N100
▨ N50-50
bc
cd
cd
ab
a
a
cd
d
e
WB
WF
WW


[A]
N supply (kg N ha⁻¹)
240
200
160
120
80
40
0
CT
NT
ab
b
a
b
c
d
WB
WF
WW
[B]
Ndfs (kg N ha⁻¹)
200
170
140
110
80
50
CT
NT
ab
b
a
b
c
d
WB
WF
WW
[C]
¹⁵N REC (%)
50
40
30
20
10
CT
NT
a
b
a
b
b
c
WB
WF
WW

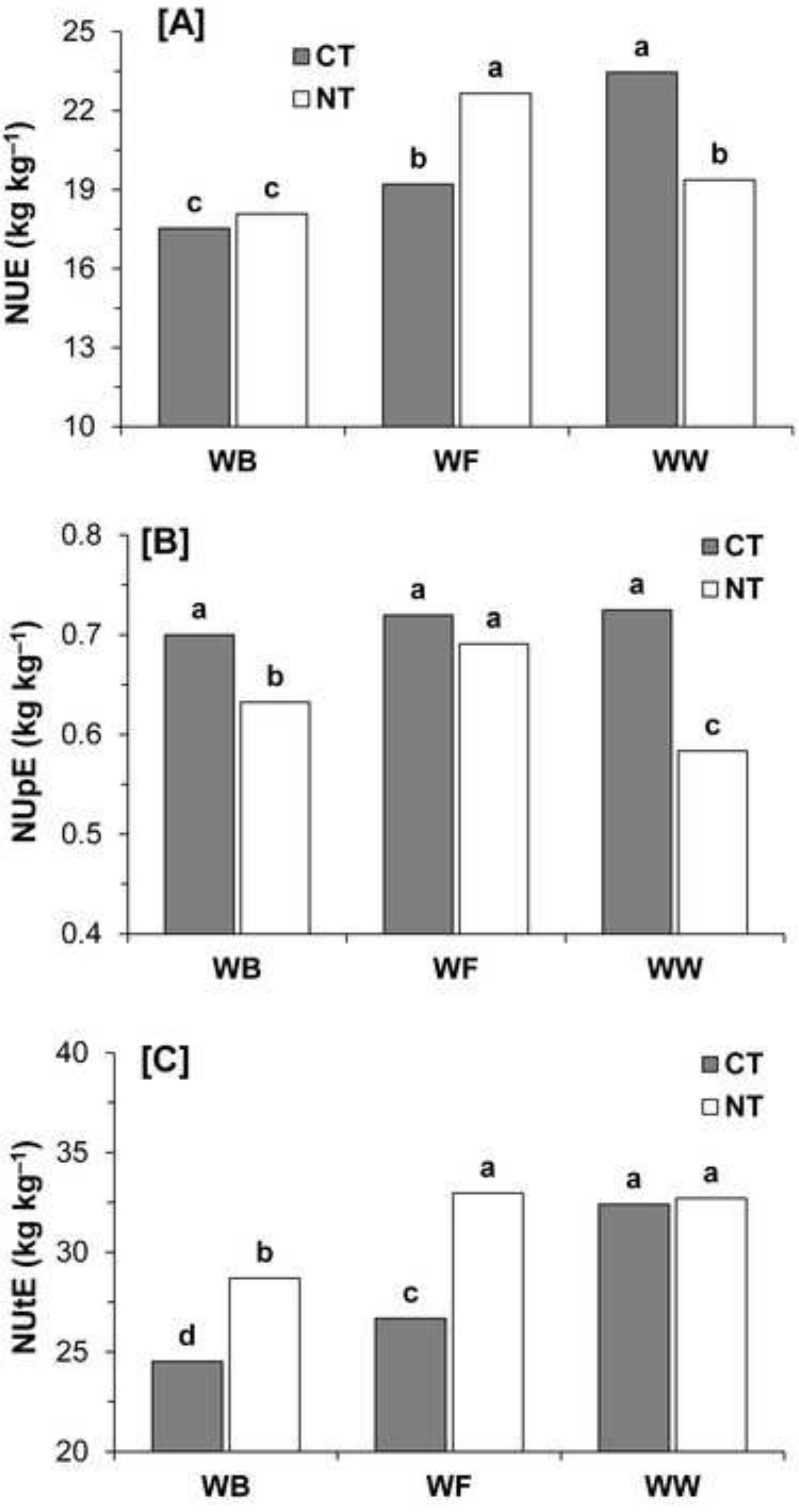

[A]
■ CT
□ NT
NUE (kg kg$^{-1}$)
25
22
19
16
13
10
c
c
b
a
a
b
WB
WF
WW
[B]
■ CT
□ NT
NUpE (kg kg$^{-1}$)
0.8
0.7
0.6
0.5
0.4
a
b
a
a
a
c
WB
WF
WW
[C]
■ CT
□ NT
NUtE (kg kg$^{-1}$)
40
35
30
25
20
d
b
c
a
a
a
WB
WF
WW

Figure8