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Long-term effects of no tillage treatment on soil N availability, N uptake, and 15N-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 15N-fertilizer recovery of durum wheat differ in relation to crop sequence / Ruisi, P., Saia, S., Badagliacca, G., Amato, G., Frenda, A.S., Giambalvo, D., DI MICELI, G.. - In: FIELD CROPS RESEARCH. - ISSN 0378-4290. - 189:(2016), pp. 51-58. [10.1016/j.fcr.2016.02.009]

Availability:

This version is available at: <https://hdl.handle.net/20.500.12318/46849> since: 2020-11-16T17:21:57Z

Published

DOI: <http://doi.org/10.1016/j.fcr.2016.02.009>

The final published version is available online at: <https://www.sciencedirect>.

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Ruisi P., Saia S., Badagliacca G., Amato A., Frenda A.S., Giambalvo D., Di Miceli G., 2016. Long-term effects of no tillage treatment on soil N availability, N uptake, and 15N-fertilizer recovery of durum wheat differ in relation to crop sequence. Field Crops Research, Volume 189, Pages 51-58, ISSN: 0378-4290

which has been published in final doi <https://doi.org/10.1016/j.fcr.2016.02.009>
(<https://www.sciencedirect.com/science/article/abs/pii/S0378429016300351>)

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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 ¹⁵N-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 ¹⁵N-fertilizer recovery. This reduction in uptake was markedly more evident in WW than in WF or WB, and when all of the ¹⁵N-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, ¹⁵N-fertilizer recovery, Mediterranean environment

Abbreviations: NT, no tillage; CT, conventional tillage; NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; % ¹⁵N_{REC}, percentage of ¹⁵N-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

1 recovery, although the differences between the two tillage techniques can vary widely in relation to
2 the method of N-fertilizer placement (e.g., broadcast, sidebanding, below seed row), N sources, and
3 N fertilization timing (Malhi et al., 2001). Conversely, under reduced tillage or NT, N
4 mineralization rates can be increased compared to plowed systems as a result of an increase in the
5 amount of both soil organic carbon and soil organic N pools, and an intensification of soil microbial
6 activity (Sharifi et al., 2008). Moreover, surface crop residues help to conserve soil moisture and
7 mitigate extreme soil temperatures, thereby generating favorable conditions for soil microbial
8 activity during dry years and consequently increasing soil N mineralization (van Donk et al., 2010;
9 Moreno-Cornejo et al., 2014). Obviously, many factors can be responsible for discrepancies in soil
10 N availability under CT and NT, including climatic conditions, soil characteristics, management
11 practices, and the duration of experiments. For example, some studies have reported that the effects
12 of tillage system on N cycling can vary markedly according to crop sequence due to a synergistic
13 effect of these two factors on soil organic matter, water retention, and microbial activity (López-
14 Bellido et al., 1997; Lafond et al., 2006; Pala et al., 2007; Melero et al., 2011; Amato et al., 2013).
15 To shed light on the discussion regarding the effects of tillage system on N dynamics, N
16 availability, and N-fertilizer recovery, and considering the limited number of studies conducted in
17 Mediterranean areas on this topic, we performed a 2-year study to verify whether, and if so to what
18 extent, the continuous application of NT affects soil N availability, recovery of ¹⁵N-labeled
19 fertilizer, and N use efficiency (NUE) and its components (N uptake efficiency, NUpE; N
20 utilization efficiency, NUtE). We used durum wheat as the focal crop, and conducted the study
21 within a long-term experiment during which NT was continuously applied for over 15 years. We
22 also varied both the crop sequence and timing of N fertilization. Our results will be useful for
23 helping farmers to plan correct N fertilization strategies to increase the sustainability of cropping
24 systems by improving the use efficiency of both native and auxiliary resources.

2. Materials and methods

2.1. Site characteristics

51 A long-term field experiment was started in the 1991–1992 growing season at Pietranera farm,
52 which is located about 30 km north of Agrigento, Sicily, Italy (37°30'N, 13°31'E; 178 m a.s.l.), on
53 a deep, well-structured soil classified as a Chromic Haploxerert (Vertisol). Soil characteristics
54 (measured at the beginning of the experiment and referring to the 0–0.40 m layer) were as follows:
55 52.5% clay, 21.6% silt, 25.9% sand, pH 8.1 (1:2.5 H₂O), 1.40% total C (Walkley Black), 1.29 g kg⁻¹
56 total N (Kjeldahl), 36 mg kg⁻¹ available P (Olsen), 340 mg kg⁻¹ K₂O (exchangeable potassium),
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1 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
2 $0.16 \text{ cm}^3 \text{ cm}^{-3}$ permanent wilting point (pF 4.5). The climate of the experimental site is semiarid
3 Mediterranean, with a mean annual rainfall of 581 mm, mostly in autumn-winter (74%) and in
4 spring (18%). The dry period is from May to September. The mean air temperature is 15.9°C in
5 autumn, 9.8°C in winter, and 16.5°C in spring. The average minimum and maximum annual
6 temperatures are 10.0°C and 23.3°C , respectively. The weather data were collected from a weather
7 station located within 500 m of the site.
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11 2.2. *Experimental design and crop management*

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13 The experiment was set up as a strip-plot design with two replications. Three soil tillage systems
14 (CT, NT, and reduced tillage [RT]) served as vertical treatments and three crop sequences
15 (continuous wheat, WW; wheat–faba bean, WF; and wheat–berseem clover, WB) served as
16 horizontal treatments. CT consisted of one moldboard plowing to a depth of 0.30 m in the summer,
17 followed by one or two shallow hallowing operations before planting. In the RT plots, primary
18 tillage consisted of chisel plowing to a depth of 0.40 m (non-inverting action) in the summer
19 followed by moldboard plowing to a depth of 0.15 m (after the first rains of autumn) and followed
20 by one shallow harrowing operation to prepare a proper seedbed: the moldboard plowing operation
21 was omitted beginning in the eighth year of the experiment (1998–1999). Finally, NT consisted of
22 sowing by direct drilling. Wheat was planted in rows spaced 0.16 m apart (for all tillage
23 treatments), always at 350 viable seeds m^{-2} , while faba bean and berseem clover were sown at 40
24 and 1200 viable seeds m^{-2} , respectively, with an inter-row spacing of 0.75 m for faba bean and 0.16
25 m for berseem clover. Cultivation and tillage treatments were performed with commercial farm
26 equipment. The plot size was 370 m^2 ($18.5 \times 20.0 \text{ m}$). Each year, both rotations (WF and WB) were
27 duplicated in reverse order so as to obtain data annually from all crops. In NT plots, weeds were
28 controlled with glyphosate (N-[phosphonomethyl] glycine) before planting at a dose of 533–1066 g
29 a.e. ha^{-1} , depending on the development of weeds. During the wheat growing season, weeds were
30 controlled by applying post-emergence herbicides at the early growth stage of the crop, with no
31 differences among the three tillage systems. In the faba bean plots, weeds were controlled
32 mechanically by shallow hoeing. Berseem clover and faba bean were harvested in June or July each
33 year, leaving standing straw; loose residues were spread uniformly throughout the plot. Wheat
34 stubble (about 20–25 cm from the soil surface) was left standing and the straw was baled and
35 removed from the field. The soil surface was covered by mulch in the NT treatments, and the
36 coverage was always $>30\%$. More details on how the trial was performed are reported in
37 Giambalvo et al. (2012) and Amato et al. (2013).
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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 ¹⁵N-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: N₀, no N fertilizer; N₁₀₀, 100 kg N ha⁻¹ all at once, distributed at crop emergence; and N₅₀₋₅₀, 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 N₁₀₀ and N₅₀₋₅₀ subplots were labeled with ¹⁵N fertilizer [100 kg N ha⁻¹ as (NH₄)₂SO₄ 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 ¹⁵N-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 NH₄-N and NO₃-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 60°C 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 ¹⁵N enrichment. The concentrations of total N and ¹⁵N 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 ¹⁵N enrichment of biomass were used to calculate the labeled-fertilizer N recovery (¹⁵N_{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

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

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

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

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

31 **3. Results**

32 *3.1. Weather conditions*

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35 The weather conditions during the experimental period are shown in Figure 1. Total rainfall was
36 508 mm in 2005–2006, 12% lower than the long-term average for the area, and 443 mm in 2007–
37 2008, 24% lower than the long-term average. The mean monthly temperature was 16.0°C in 2005–
38 2006 and 16.9°C in 2007–2008, very similar to the long-term mean temperature (16.6°C).

39 *3.2. Biomass and grain yield, N uptake, and grain protein content*

40 Table 1 presents the results of the statistical analyses for the effects of the applied treatments and
41 their interactions on wheat yield and yield components, and N efficiency parameters. On average,
42 biomass and grain yield of durum wheat were markedly lower in WW than in WB and WF (Fig.
43 2A,B). The effects of tillage system on biomass and grain yield greatly varied by crop sequence (the
44 tillage \times crop sequence interaction was significant at $P < 0.001$). In WW, NT dramatically reduced
45 biomass and grain yield compared to CT (–36% and –37%, respectively). In WB and WF, grain
46 yield was 5% and 9% higher, respectively, under NT than CT but no differences were observed for
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1 biomass between the two tillage systems. The effects of tillage system and crop sequence on the
2 number of spikes per square meter and the number of kernels per spike were similar to those
3 observed for biomass (Fig. 3A,B). On the contrary, NT increased 1000-kernel weight compared to
4 CT in WB and WF but not in WW (Fig. 3C). NT decreased total N uptake compared to CT in all
5 crop sequences, and this reduction was stronger in WW (−38%) than WB and WF (−8% and −13%,
6 respectively; Fig. 4A). Grain protein content was lower in NT than CT in all crop sequences with a
7 stronger decrease in WF (−15%) than WB and WW (−6% and −3%, respectively; Fig. 4B).

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

25 3.3. N efficiency and recovery of ^{15}N -fertilizer

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

31 The effects of tillage system on NUE varied by crop sequence (the tillage × crop sequence
32 interaction was significant at $P < 0.001$; Table 1 and Fig. 7A). In particular, compared to CT, NT
33 increased NUE from 19.2 to 22.7 in WF but had the opposite effect in WW, decreasing it from 23.4
34 to 19.4; no differences due to the tillage system were observed in WB. On average, NT led to a
35 reduction of NUpE compared to CT but the detrimental effect varied by crop sequence, being
36 greater in WW than in WB and WF (Fig. 7B). The application of NT instead of CT led to an
37 increase in NUtE only in WB and in WF, whereas no significant effect was observed in WW (Fig.
38 7C). The NHI was higher in NT than CT in WB and WF but not in WW (data not shown).

39 On average, N fertilization (irrespective of fertilizer timing) resulted in a 35% reduction of NUE
40 compared to the N_0 treatment, due to a reduction of both NUpE (−16%, on average) and NUtE (−
41 22%, on average). No differences were observed in the other three indices due to the N fertilizer
42 timing. Splitting the fertilizer (N_{50-50}) increased $^{15}\text{N}_{\text{REC}}$ compared to the N_{100} treatment in NT but
43 not in CT (Fig. 8).

4. Discussion

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

13
14 In the present research, N uptake was, on average, lower with NT than with CT; this result is in
15 agreement with the findings of other authors (Ishaq et al., 2001; Soon et al., 2006). Our data seem to
16 suggest that this was mainly attributable to a reduction of soil N available to plants under NT
17 compared to CT, and only in small part to a lower efficiency in N uptake (i.e., in the NUpE).
18 According to Silgram and Shepherd (1999), soil cultivation can enhance the decomposition of
19 organic matter by altering the structure, temperature, and aeration of soil, as well as the distribution
20 of crop residues along the soil profile and the degree of physical protection of organic matter from
21 microorganisms or their enzymes. Watson et al. (2002) found that tillage stimulates microbial
22 activity in the soil, which increases N availability. Other authors have emphasized that a key factor
23 reducing soil N under NT conditions compared to CT is the presence of crop residues on the soil
24 surface, which can increase the N immobilization rate, thus decreasing its availability to the crop
25 (Erenstein, 2002; Dawson et al., 2008; Giller et al., 2009). In this study, the differences found in the
26 available soil N between CT and NT were marked in the continuous wheat plot (−30% in NT
27 compared to CT) and, on the whole, they were small when wheat was grown in rotation with a
28 legume crop (−7% on average). It is possible that the retention of cereal crop residues (which have a
29 high C:N ratio, unlike legume crops) on the soil surface may have exacerbated the N stress in WW
30 by temporary N immobilization (as already highlighted by Erenstein 2002), and as a consequence,
31 led to a lower N availability for the crop.

32
33 Even though organic N mineralization rates are often higher in plowed systems, a gradual
34 accumulation of greater amounts of organic matter in NT systems over time may compensate for
35 this effect (Salinas-Garcia et al., 1997). Rice et al. (1986) suggested that the lower availability of N
36 frequently observed in NT soils can in some cases be a transient effect; in their experiment,
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1 availability of soil N in NT apparently approached that of CT after approximately 10 years.
2 However, our data on soil N availability, which were collected after 14–16 years of continuous
3 application of CT and NT, do not allow us to hypothesize on the occurrence of a transient effect.

4 In our experiment, N fertilization (irrespective of fertilizer timing) resulted in increases in wheat
5 grain yield in WW but not in WB and WF. Once again, this result can be attributable to different
6 soil N availability among the three crop sequences; in fact, soil N availability was markedly lower
7 in WW than WB and WF, and it is well known that crop responses to N fertilization are greater
8 under conditions of low soil N availability (Godard et al., 2008). The increase in soil N availability
9 (calculated in N₀ plots on the basis of N uptake by wheat and the amount of residual N in soil at
10 harvest) in WB and WF compared to WW was on average 60–70 kg N ha⁻¹. This result is not
11 surprising, as other studies (Hauggaard-Nielsen et al., 2009; Giambalvo et al., 2011) have already
12 shown that faba bean and berseem clover are both able to fix high amounts of atmospheric N (even
13 close to 300 kg N ha⁻¹), which will then obviously become partly available to the subsequent crop.

14 We observed a significant decrease in the percentage of ¹⁵N-fertilizer recovery (% ¹⁵N_{REC}) with the
15 NT technique versus the CT method. This may be attributable to the higher N losses from soil
16 through volatilization under NT compared to CT, due to the lack of incorporation of N fertilizer into
17 the soil (Fox and Piekielek, 1993; Angás et al., 2006). Moreover, the increased potential for N
18 immobilization at the surface of no-till soils may significantly reduce crop recovery of N fertilizer
19 (Rice and Smith, 1984). Considering that this potential for N immobilization varies in relation to the
20 composition of crop residues (proportionally to the C:N ratio), this would also explain why in our
21 experiment the differences in the % ¹⁵N_{REC} between CT and NT were higher in WW than in both
22 WB and WF. Moreover, our data indicated improved % ¹⁵N_{REC} when N application was split in NT
23 systems but not in CT. This could be explained considering that a greater N immobilization
24 generally occurs under NT with respect to CT during the early growth stages of a crop, as
25 previously observed by other authors (Haugen Kozyra et al., 1993; Melaj et al., 2003). Therefore,
26 splitting N fertilization into two fractions (i.e., in our case, applying 50% at crop emergence and
27 50% at the end of tillering) would reduce the risk of N immobilization under NT conditions, thus
28 increasing the chances for this element to be taken up by the crop. Furthermore, the excess soil
29 water that often accumulates in no-till soils during winter (the rainy season in the Mediterranean
30 environment), when plants are in the early growth stages, along with the lower soil temperatures
31 compared to plowed soils, can slow down initial crop growth, with negative repercussions on N
32 uptake; this, in turn, leads to a greater chance that N will be released into the environment. On the
33 contrary, in soils managed with the CT technique, plants often have a more rapid early growth
34 (which is favored by the higher soil temperatures and the faster drainage compared to no-till soils);
35 this would imply higher N requirements, and therefore, a greater chance that the crop will absorb
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1 the N provided by the fertilizer. The higher number of spikes per square meter we observed in CT
2 compared to NT seems to confirm that soil cultivation (i.e., CT) led to more favorable conditions
3 for tillering and plant growth during the vegetative phase of the cycle. The abovementioned
4 negative effects on initial crop growth due to NT application are generally widely counteracted by
5 the greater availability of water for the crop during the spring, attributable to reduced soil water
6 evaporation with NT (Blevins and Frye, 1993; Lampurlanés and Cantero-Martínez, 2006).
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9 A meta-analysis by Lundy et al. (2015) evaluated the influences of crop and environmental
10 variables on no-till productivity, and found that N fertilization reduces yield declines following no-
11 till adoption and that this effect is more marked in tropical/subtropical regions than in temperate
12 regions. Our results disagree with their findings, as the interaction of tillage system \times N fertilization
13 was not significant for grain yield. On the other hand, we did not observe a yield decline following
14 NT adoption when a legume crop was included in the crop sequence; that is, a marked yield decline
15 due to the adoption of NT was observed only in the continuous wheat plot. Therefore, our data
16 suggest that the yield decline related to the adoption of NT in WW must be attributed to factors
17 beyond N deficiency, which could be overcome by increasing N-fertilizer rate (as suggested by
18 Alvarez and Steinbach, 2009); such factors may include an increased occurrence of crop diseases,
19 increased weed competition, and so forth. This seems more plausible when we consider that the
20 wheat monoculture included in this study has been practiced continuously for over 15 years.
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23 Considering the lower N availability associated with NT compared to CT systems as well as the
24 lower efficiency of N-fertilizer utilization observed under NT soil, it is not surprising that, on
25 average, a lower grain protein content was found with NT than with CT. This result agrees with the
26 findings of López-Bellido et al. (1998) and McConkey et al. (2002). Finally, we found that the
27 lower grain protein content obtained with NT was particularly accentuated when wheat was grown
28 after a legume crop (in both WF and WB plots), whereas the differences between NT and CT were
29 smaller in WW. This result may be associated with the effects of tillage on wheat grain yield and N
30 uptake, which both varied by crop sequence.
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33 Tillage system affected the N efficiency indices (NUE, NUpE, and NUtE) differently according to
34 crop sequence. Under WW, the application of NT led to a decrease in NUE attributable to a
35 reduction in grain yield from CT to NT that was much higher than that observed for N supply. This
36 suggests that, in WW, reductions in grain yield observed in NT compared to CT were due not only
37 to changes in N supply but also to other factors. In this regard, an increase in the incidence of some
38 pathogens of wheat (*Gaeumannomyces graminis*, *Rhizoctonia solani*, *Pythium* spp., and other
39 species belonging to the genus *Fusarium*) that we noticed in plants grown under NT compared to
40 CT may have played an important role in the WW system. On the other hand, it has already been
41 shown that when NT and continuous wheat (retaining wheat residues on the soil surface) are
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1 combined, some residue-borne pathogens can progressively become extremely problematic (Bockus
2 and Shroyer, 1998; Paulitz et al., 2002). This hypothesis would also explain the reduction of NuPE
3 found in WW (but not in WB and WF) due to the application of NT.

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

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

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44 This work was funded by the Italian Ministry of Agriculture (project SICOBIOBOS).
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Figure legends

1 Fig. 1. Accumulated rainfall (A) and 10-day mean air temperature (B) at the experimental site
2 during the two growing seasons (2005–2006 and 2007–2008); 30-year average 10-day temperatures
3 and accumulated rainfall are also included.
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6 Fig. 2. Biomass [A] and grain [B] yields of wheat grown under conventional tillage (CT) and no
7 tillage (NT) approaches in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba
8 bean; WW, continuous wheat). Different letters denote significant differences ($P < 0.05$). Data are
9 means over 2 years.
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11 Fig. 3. Grain yield components (number of spikes per square meter [A]; number of kernels per spike
12 [B]; 1000-kernel weight [C]) of wheat grown under conventional tillage (CT) and no tillage (NT) in
13 three crop sequences (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous wheat).
14 Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.
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17 Fig. 4. Nitrogen uptake [A] and grain protein content [B] of wheat grown under conventional tillage
18 (CT) and no tillage (NT) in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba
19 bean; WW, continuous wheat). Different letters denote significant differences ($P < 0.05$). Data are
20 means over 2 years.
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23 Fig. 5. Biomass [A] and grain [B] yields of wheat as affected by N fertilization (no N fertilizer, N_0 ;
24 100 kg N ha^{-1} all distributed at crop emergence, N_{100} ; 100 kg N ha^{-1} split distribution, using 50% at
25 crop emergence and 50% at the end of tillering, N_{50-50}) in three crop sequences (WB, wheat–
26 berseem clover; WF, wheat–faba bean; WW, continuous wheat). Different letters denote significant
27 differences ($P < 0.05$). Data are means over 2 years.
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31 Fig. 6. Amount of potentially available N in soil for crops (N supply [A], determined on sub-plots
32 with no N applied), amount of N derived from soil (Ndfs [B]), and recovery of ^{15}N -fertilizer ($^{15}\text{N}_{\text{REC}}$
33 [C]) in wheat grown under conventional tillage (CT) and no tillage (NT) in three crop sequences
34 (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous wheat). Different letters
35 denote significant differences ($P < 0.05$). Data are means over 2 years.
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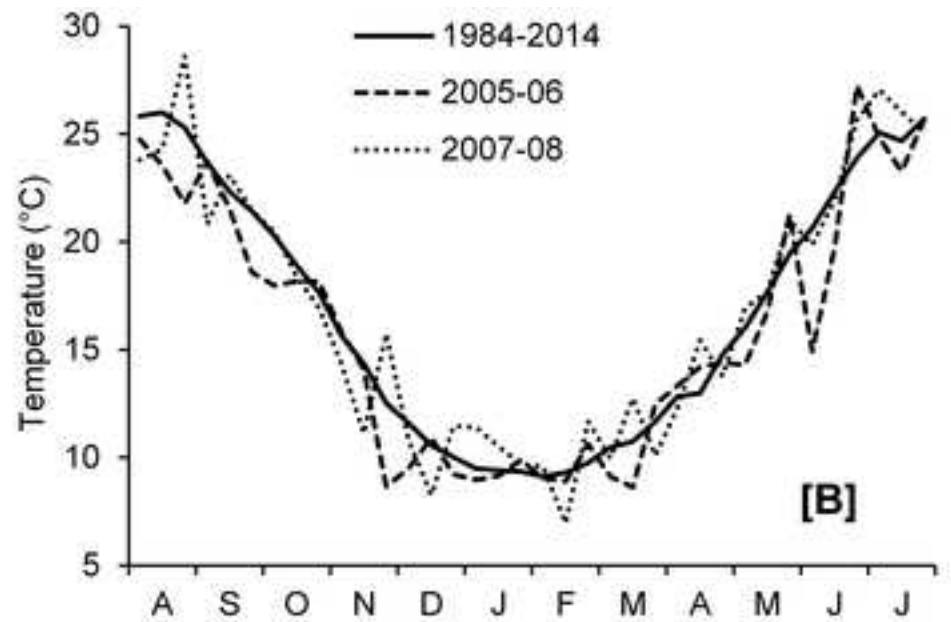
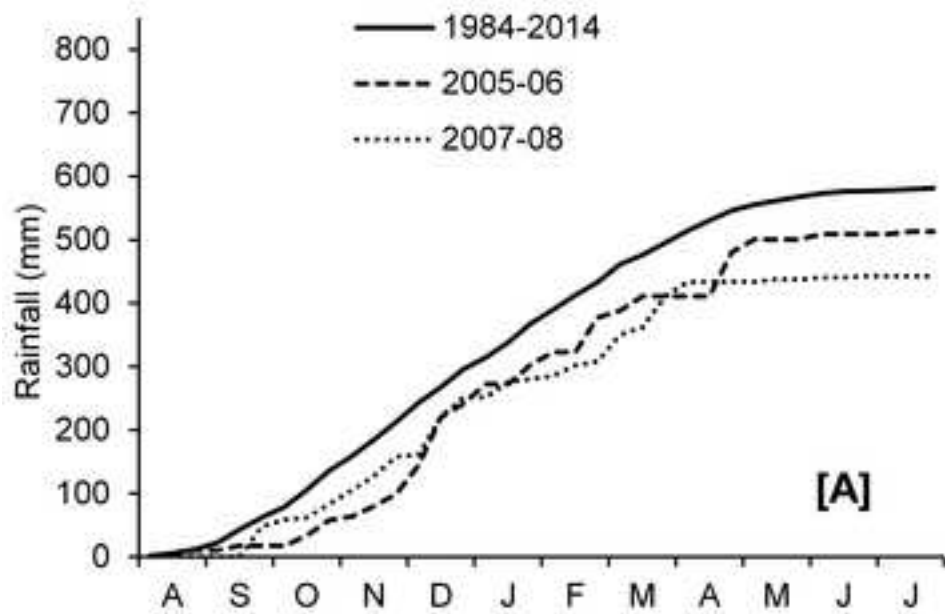
39 Fig. 7. Nitrogen efficiency indices (N use efficiency, NUE [A]; N uptake efficiency, NUpE [B]; N
40 utilization efficiency, NUtE [C]) in wheat grown under conventional tillage (CT) and no tillage
41 (NT) in three crop sequences (WB, wheat–berseem clover; WF, wheat–faba bean; WW, continuous
42 wheat). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.
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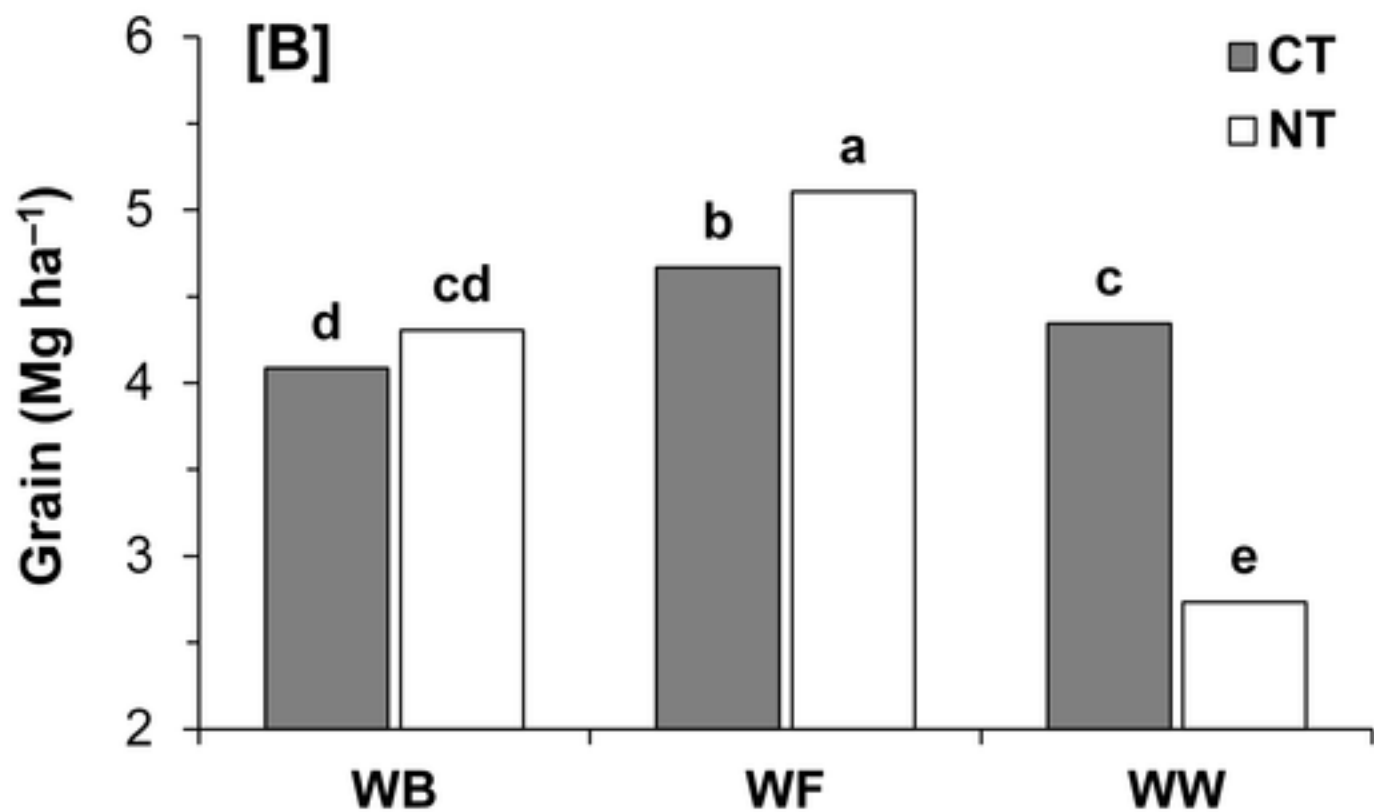
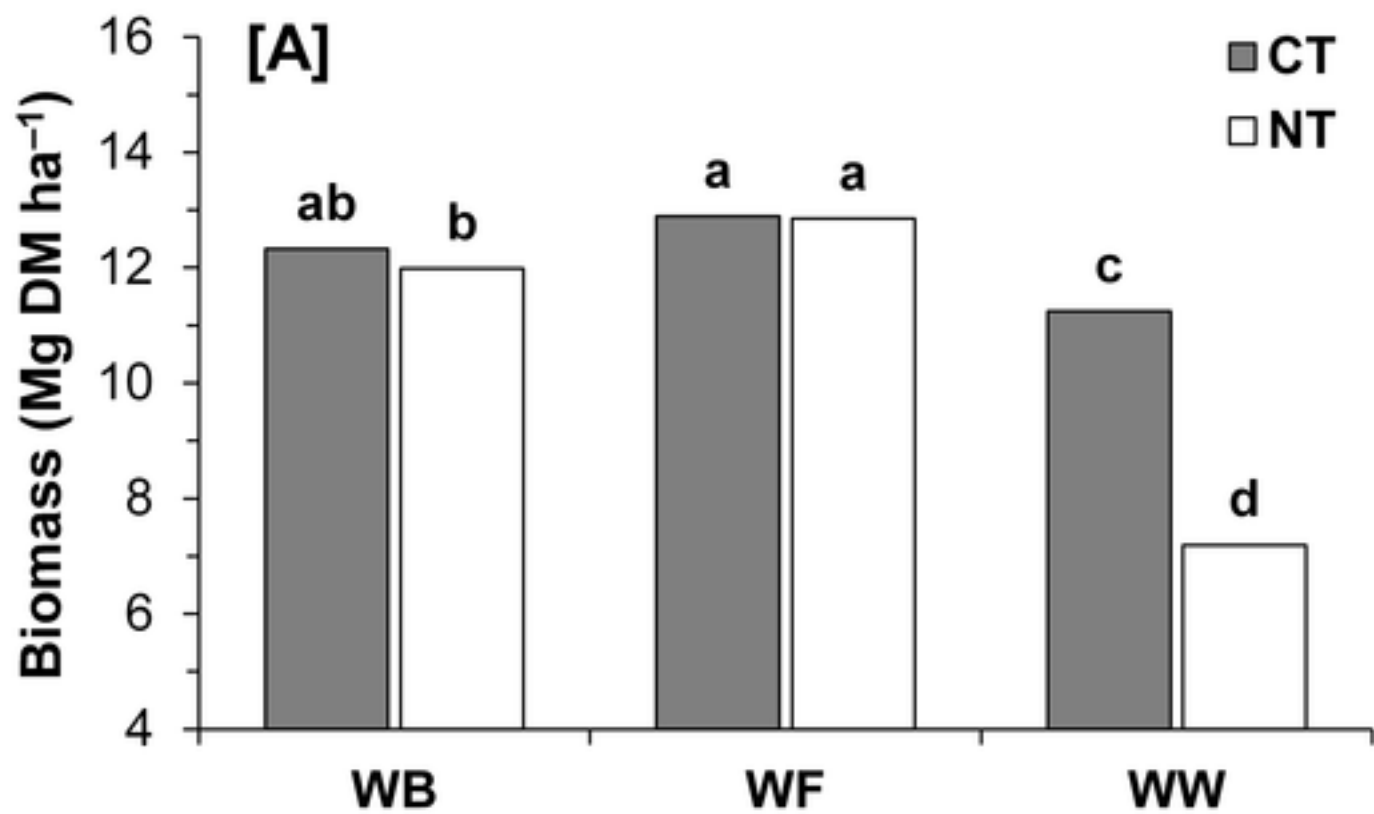
45 Fig. 8. Recovery of ^{15}N -fertilizer ($^{15}\text{N}_{\text{REC}}$) by wheat as affected by tillage system (conventional
46 tillage, CT; no tillage, NT) and N fertilization (100 kg N ha^{-1} all distributed at crop emergence,
47 N_{100} ; 100 kg N ha^{-1} split distribution, using 50% at crop emergence and 50% at the end of tillering,
48 N_{50-50}). Different letters denote significant differences ($P < 0.05$). Data are means over 2 years.
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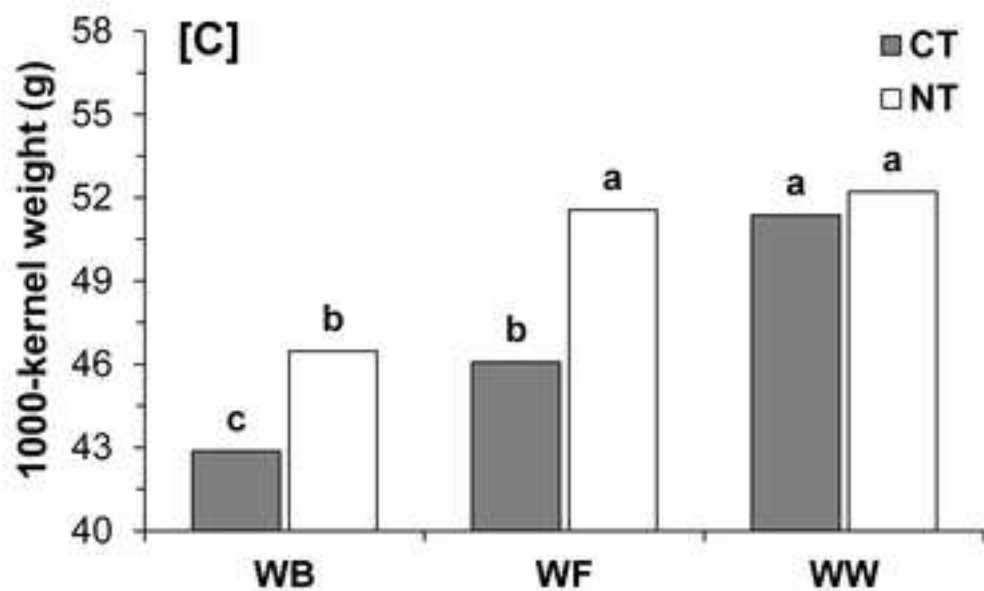
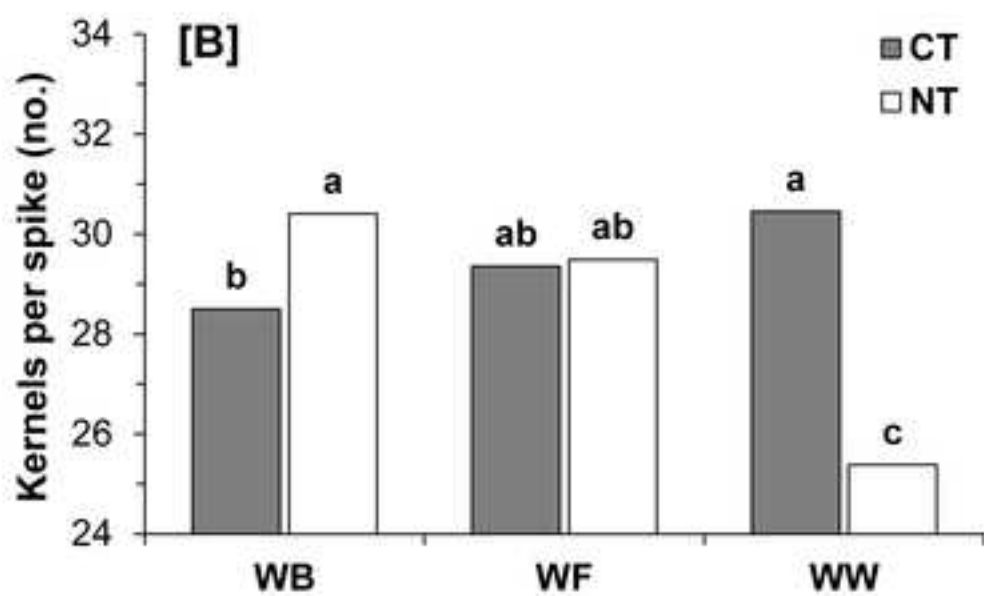
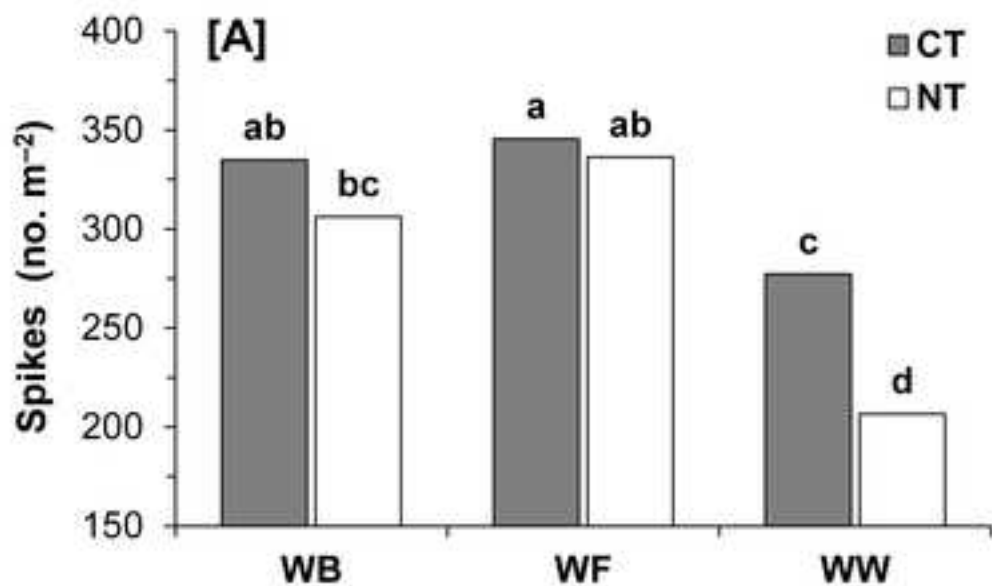
Table 1

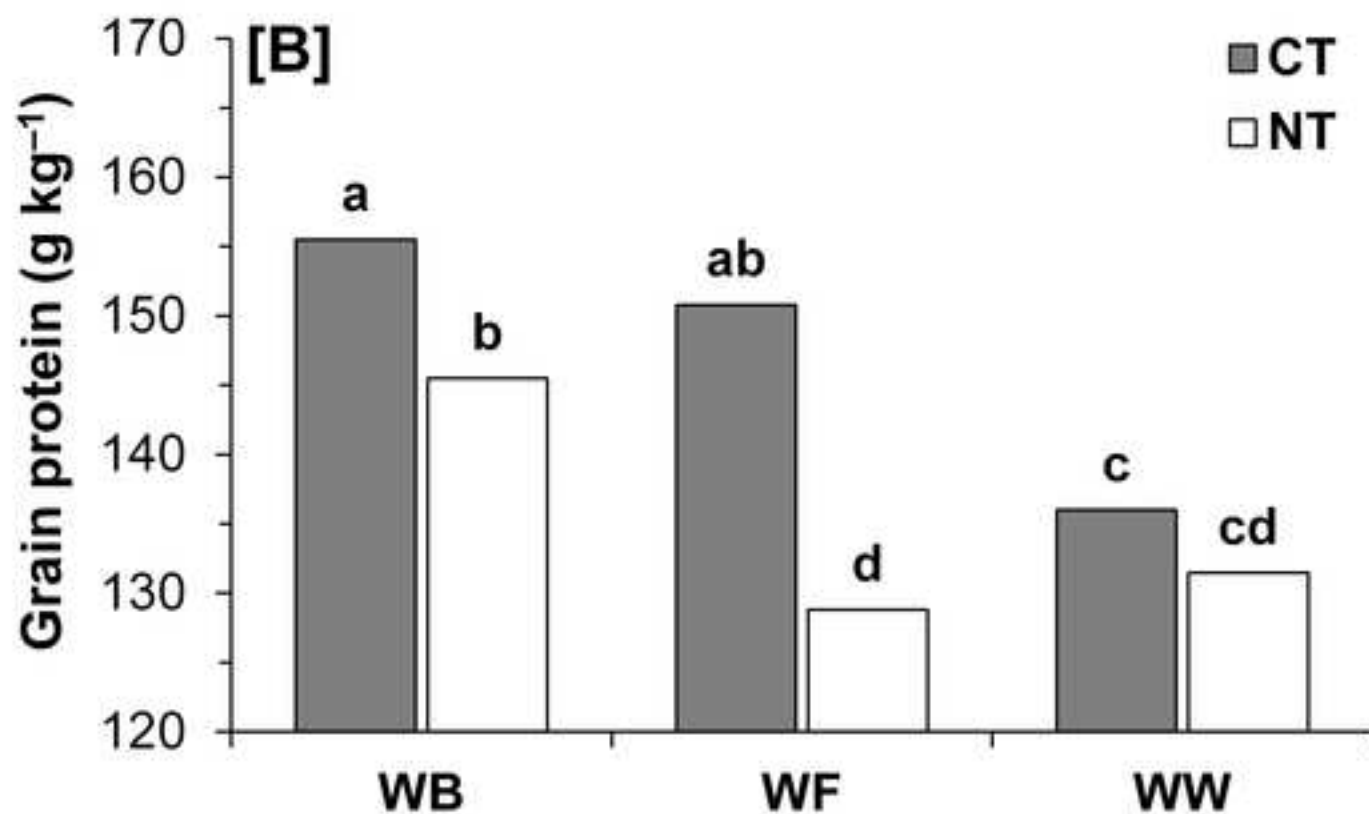
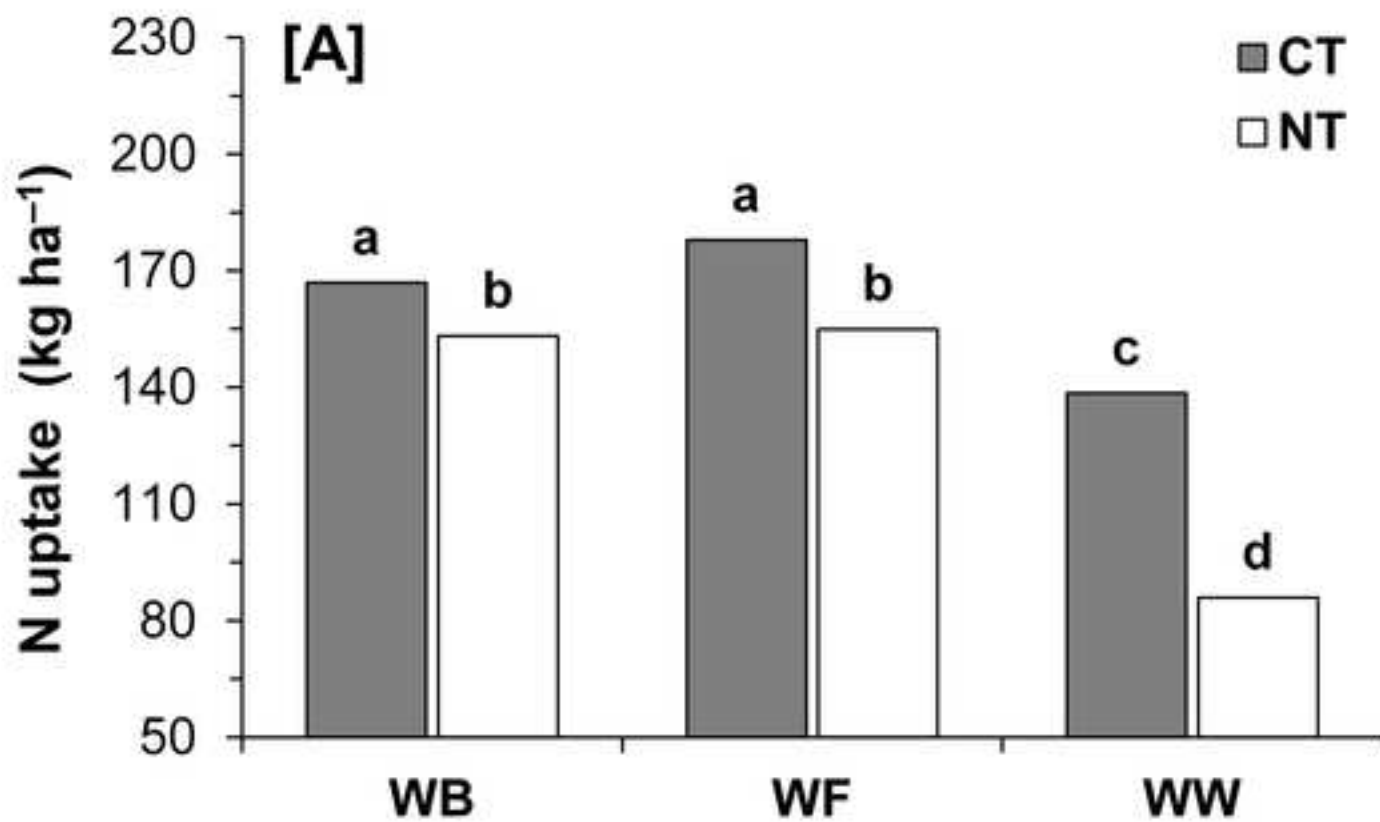
Table 1. *P*-values for the effects of crop sequence, tillage system, and N fertilization on yield and yield components, N uptake, grain protein content, potentially available N in soil for crops (N supply), and N efficiency parameters (Ndfs, nitrogen derived from soil; % ¹⁵N_{REC}, labeled-fertilizer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

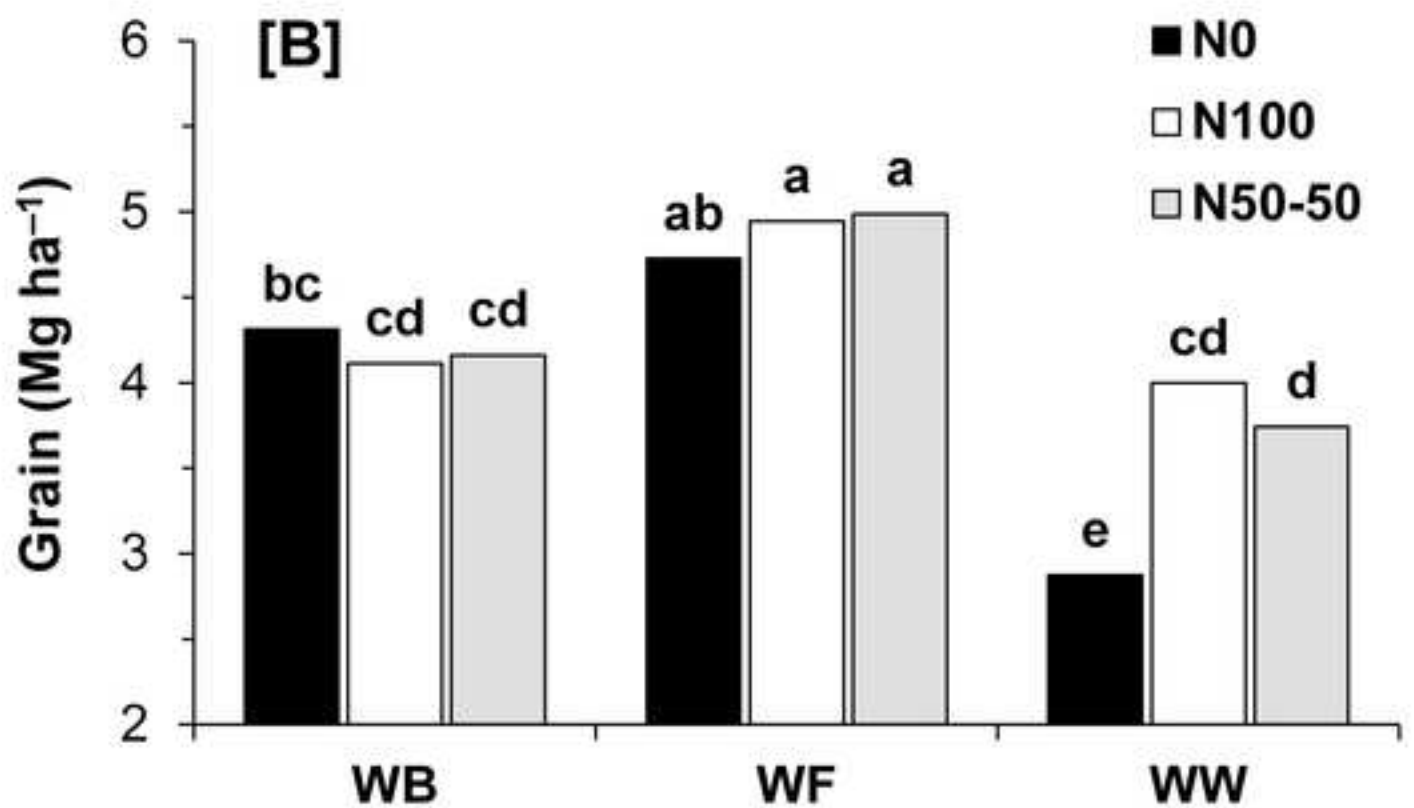
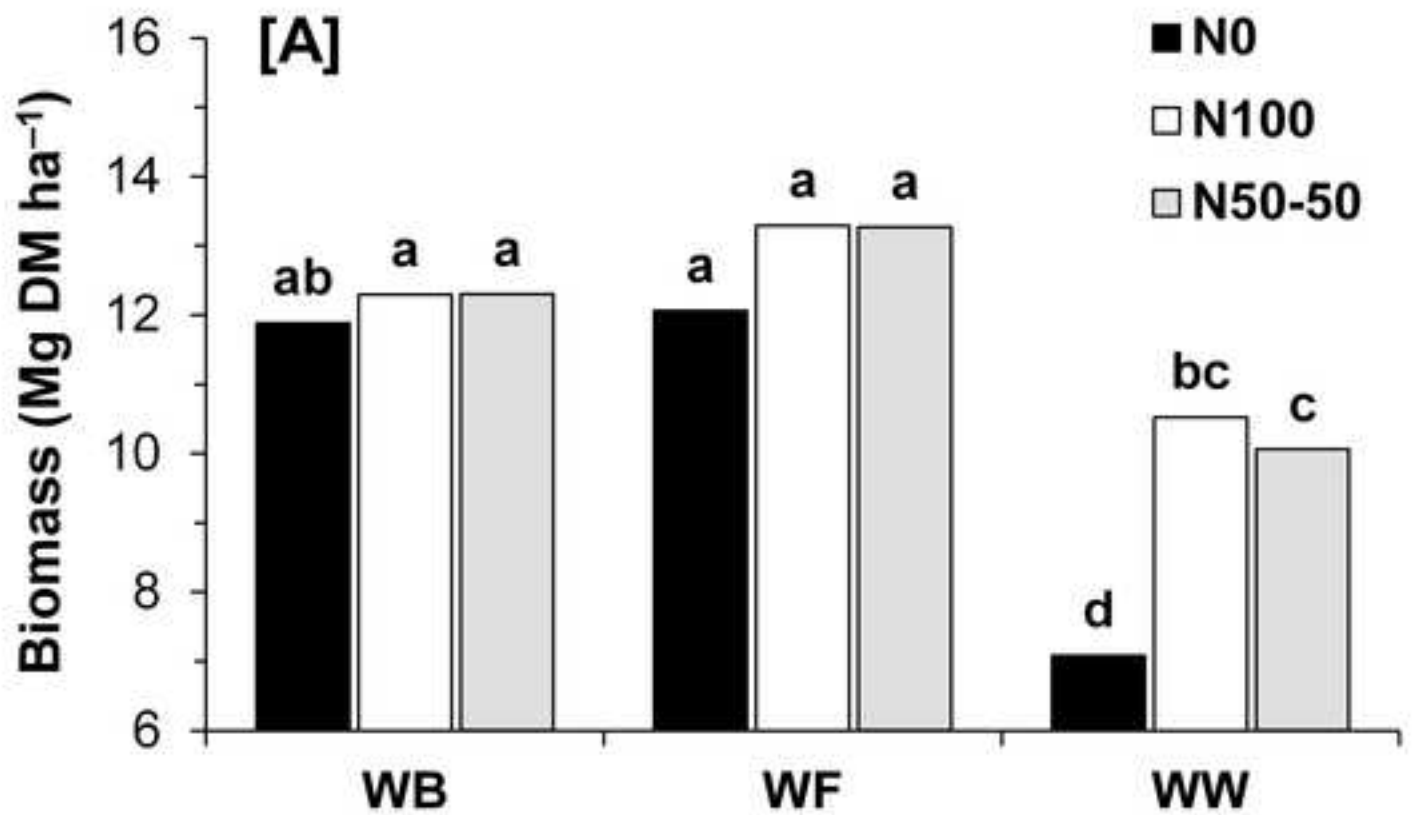
	Mean effects			Interactions			
	Crop sequence (CS)	Tillage (T)	Fertilization (F)	CS × T	CS × F	T × F	CS × T × F
Traits	<i>P</i> -value						
Biomass	<0.001	<0.001	<0.001	<0.001	0.049	ns	ns
Grain	<0.001	<0.001	ns	<0.001	<0.001	ns	ns
No. spikes m ⁻²	<0.001	<0.001	ns	0.033	ns	ns	ns
Kernels per spike	0.007	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

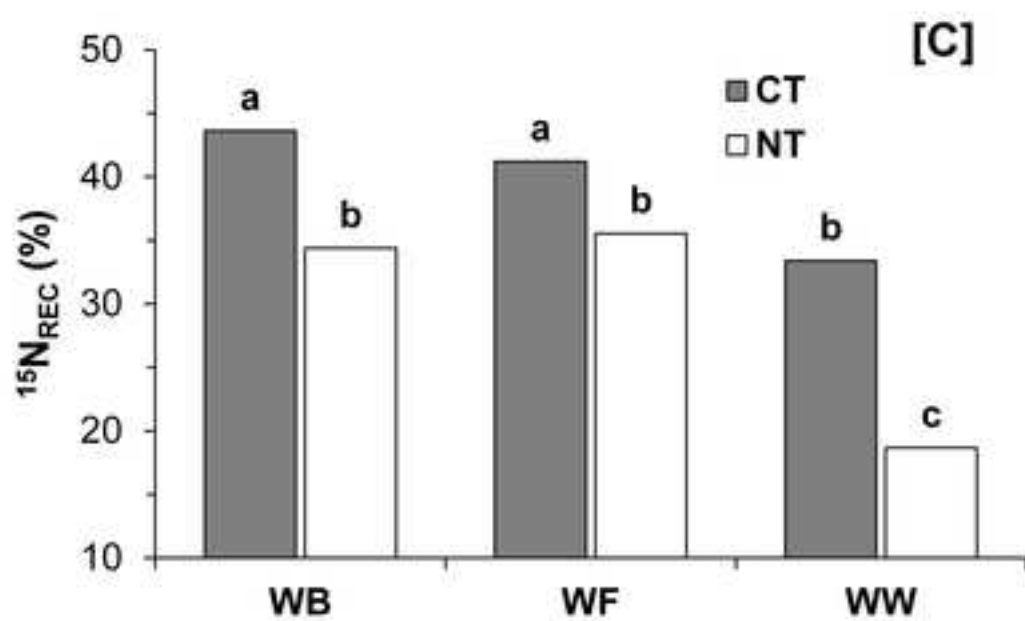
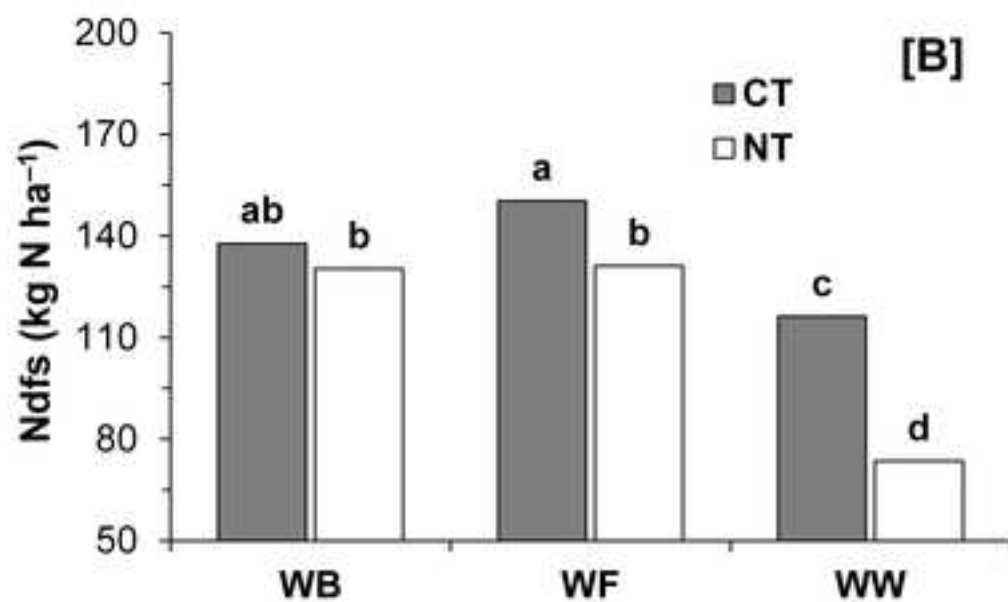
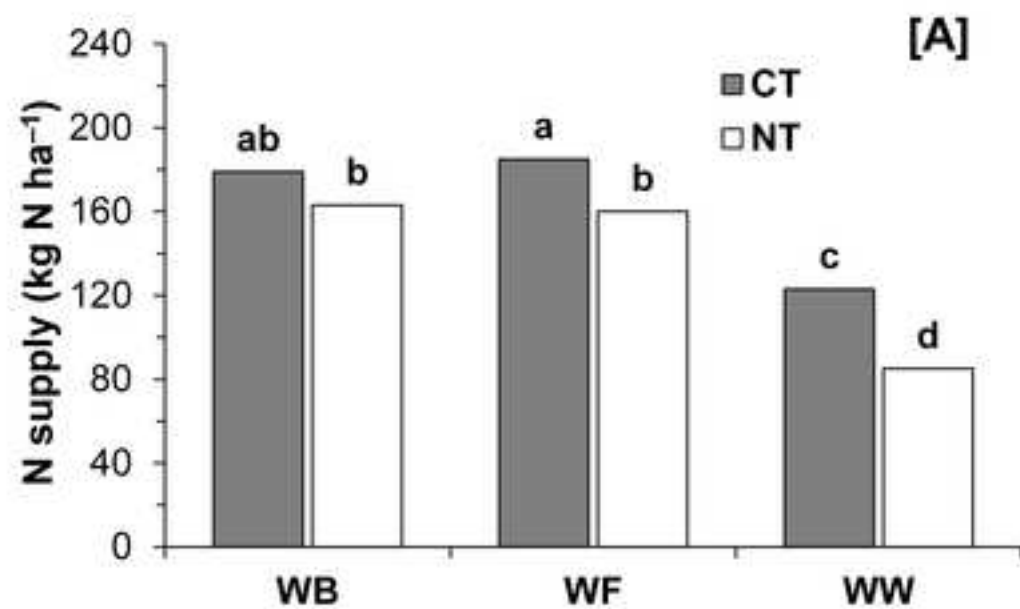












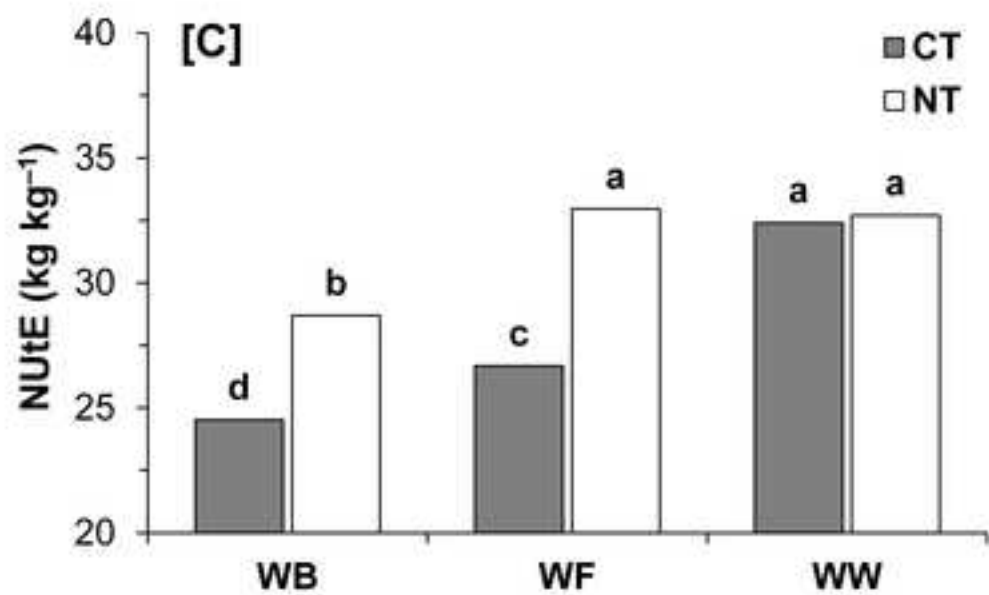
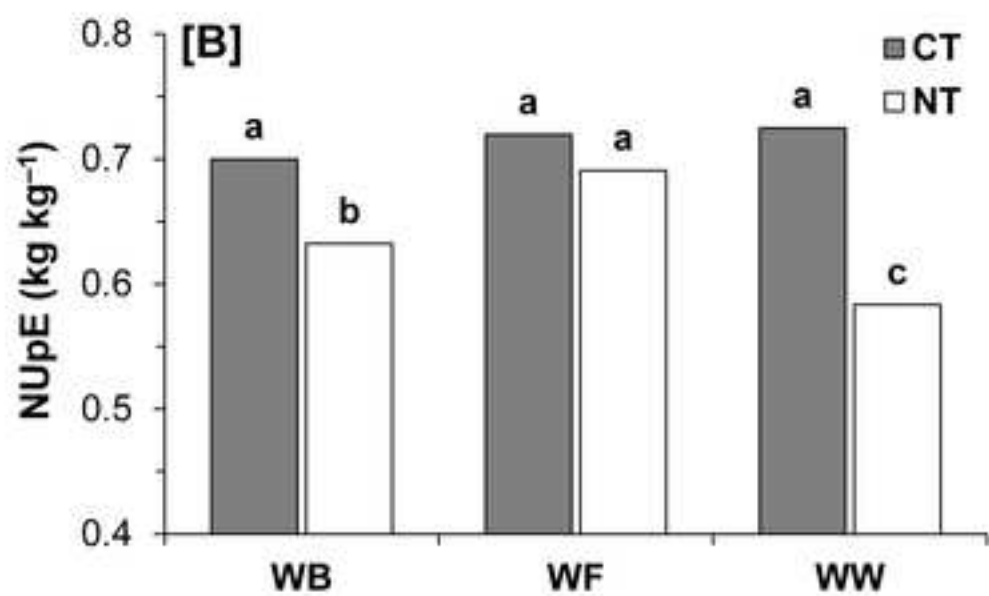
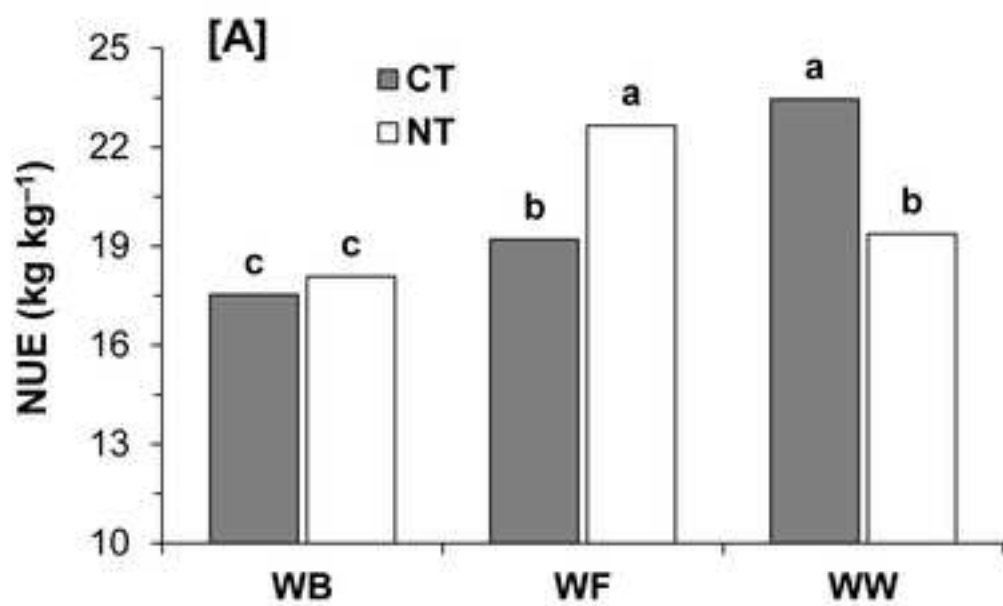


Figure8
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