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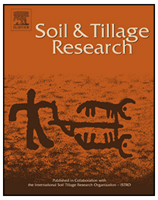

Tillage effects on labile pools of soil organic nitrogen in a semi-humid climate of Argentina: A long-term field study

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ABSTRACT

Tillage systems strongly affect nitrogen (N) mineralization. However, there is still only limited information on the relationship between N in labile soil organic matter (SOM) fractions and crop N uptake under different tillage systems in areas with poor water availability. This study discusses the long-term effect of two tillage systems on i) the N-content in labile organic matter fractions and their relationship with the N mineralization potential at three depths (0–5; 0–10 and 0–20 cm), ii) the factors that affect the N mineralization potential, and iii) the relationship between potentially mineralizable N (N_0) and crop N uptake in a semi-humid climate. In a long-term experiment, a Typic Argiudoll was sampled under two contrasting tillage systems: no-tillage (NT) and conventional tillage (CT). The soil sampling was performed over four years of the crop sequence (2003, 2009, 2010 and 2011) when the plots were sown with winter wheat (*Triticum aestivum* L.). They were analyzed for N_0 in the form of anaerobic N, soil organic nitrogen (SON), physically separated SOM fractions and crop N uptake. Higher values of SON and labile soil N fractions were observed under NT at all three depths. Significant differences in N_0 were found between the tillage systems, with greater values under NT. Significant ($P < 0.05$) and positive correlations between N_0 and fine particulate organic carbon (fPOM-C) ($r \geq 0.66$) were found in CT and in NT at the three depths, whereas highly significant ($P < 0.001$) and negative relationships between N_0 and fine particulate organic N (fPOM-N) ($r \geq -0.83$) were found under both tillage systems at 0–5 and 0–10 cm. The most pronounced difference in these relationships between tillage systems was observed at the 0–5 cm soil depth. Significant correlations of N_0 with residue input from previous crops and the fallow period were observed under both tillage systems and for all three depths. Regarding the relationships between N_0 and wheat N uptake, no significant correlations were found for any tillage system or depth. Soil organic N fractions were shown to be strongly influenced by the residue input from the previous crop and by variable weather conditions during the fallow period. The higher content of SON fractions under NT was associated with a higher N mineralization potential, however, it did not result in increased N availability and N uptake by wheat, because of climatic conditions during the crop growing season.

1. Introduction

Nitrogen (N) is a major yield-limiting nutrient in agricultural areas worldwide (Fageria and Baligar, 2005). Enhanced N-use efficiency is fundamental (Sainz Rozas et al., 2011), due to the high cost of N-fertilizers. Tillage systems affect N mineralization and concentration in the soil through short- and long-term effects on physical, chemical, and biological properties of the soil (Sharifi

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Abbreviations: NT, no-tillage; CT, conventional tillage; N, nitrogen; N_0, potentially mineralizable N; Nan, anaerobic nitrogen; SOM, soil organic matter; POM, particulate organic matter; POM-C, particulate organic carbon; SOC, soil organic carbon; SON, soil organic nitrogen; CN, carbon-nitrogen ratio in whole soil; cPOM-C, coarse particulate organic carbon; cPOM-N, coarse particulate organic nitrogen; fPOM-C, fine particulate organic carbon; fPOM-N, fine particulate organic nitrogen; cPOM-CN, carbon-nitrogen ratio of the coarse particulate organic matter; fPOM-CN, carbon-nitrogen ratio of the fine particulate organic matter; NH_4-N, ammonium N; NO_3-N, nitrate N; Pe, extractable phosphorus; BD, bulk density; Z22, wheat tillering stage; CWR, crop water requirement.

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et al., 2008). Conventional tillage (CT) favors residue and soil organic matter (SOM) decomposition through disruption of the soil aggregates which enhance aeration and distribute carbon (C) sources more uniformly. Tillage exposes the protected organic compounds to the action of microorganisms and thus hastens C and N cycling (Mikha and Rice, 2004). Also, incorporation of crop residues results in faster turnover rates (St. Luce et al., 2011). In contrast, no-tillage (NT) promotes SOM stratification, with a higher concentration at the soil surface where residues accumulate over time (Ferreira et al., 2013). This SOM increase under NT is closely related to crop production (Diovisalvi et al., 2008), and the N released from residues depends on its N content and C:N ratio (Vigil and Kissel, 1991).

The Pampas region in Argentina is known as one of the most important grain-producing areas in the world (Satorre and Slafer, 1999), the main crops being wheat (*Triticum aestivum* L.), maize (*Zea mais* L.) and soybean (*Glycine max* L. Merr) (Reussi Calvo et al., 2013). This region includes a temperate zone known as the Humid Pampas which extends to the semi-humid zone, where rainfall is scarce and the climate is more seasonally variable. Wheat is the basic crop in production systems across a wide region of the southwestern Pampas in Argentina (Martínez et al., 2015, 2016). Considering that the wheat yield is strongly influenced by the weather conditions and soil properties, it is essential to maximize both N and water use efficiency (Galantini et al., 2004). In addition, the response to N-fertilizer application depends on the amount and distribution of rainfall in areas with low water availability (Martínez et al., 2015, 2016).

To determine optimal N-fertilization rates it is necessary to consider both the inorganic N accumulated during the fallow period and the soil organic N (SON) that is mineralized during the growing season (Sainz Rozas et al., 2011). Most estimates of soil N mineralization are based on long-term aerobic incubation (Stanford and Smith, 1972). This method determines the soil labile N fraction that can be converted to mineral forms, which is known as potentially mineralizable N (N_0). This pool can also be estimated through tests performed in the field (Bundy and Meisinger, 1994) or in laboratories by anaerobic incubation (Waring and Bremner, 1964). The anaerobic N (Nan) consists of ammonium N (NH_4-N) released by microorganisms that are killed under anoxic conditions in a soil-water slurry incubated for 7 to 14 days. The Nan was considered to be the most useful indicator of soil quality in semi-arid regions (Keeney, 1982); other authors (Bushong et al., 2007; Soon et al., 2007) have argued that Nan is the best biological indicator of N_0. Also, Reussi Calvo et al. (2013) proposed that the use of Nan to estimate N-supply through mineralization would help to better assess N-availability.

Soil organic matter and its labile fraction –particulate organic matter (POM)- are considered important factors in regulating N-dynamics (Fabrizzi et al., 2003; Gregorich et al., 2006; Cozzoli et al., 2010) in view of their key role in N-mineralization (Gómez-Rey et al., 2012) and N-availability to crops (Wander, 2004). The POM may provide more accurate information on N-mineralization as it contains easily mineralizable N-fractions. This fraction represents the mineralizable pool and it can easily predict the N-mineralization capacity (Fabrizzi et al., 2003). Haynes (2005) reported that POM is an important labile N-pool in several soils; whereas Boone (1994) found that POM contributed as little as 2–13% mineralized N. According to Galantini and Suñer (2008), the N in POM is not always directly related to mineralized N, but it may be used for N estimation. Decomposition of this labile fraction is heavily dependent on residue input and weather conditions (Galantini et al., 2014), which vary significantly between years in semi-humid areas.

In order to assess the effect of tillage systems on N-dynamics and N-availability to the crop it is important to obtain information from long-term experiments in areas with low water availability. Sharifi et al. (2008) suggested that the effect of tillage systems on active organic N might vary with the specific soil and weather conditions. In Argentina, NT systems were implemented about 40 years ago; however, few comparative studies are old enough to evaluate long-term effects, especially in Mollisols of semi-humid areas. Moraes Sá (2003) reported that conversion of CT to NT could stabilize after 20 years, which highlights the importance of long-term experiments. In Argentina, a few studies have been conducted on the relationship between N mineralization and the labile C and N fractions in the humid Pampa (Fabrizzi et al., 2003; Diovisalvi et al., 2008; Domínguez et al., 2009); however, little is known about the relationship between N in labile SOM fractions and crop N uptake under different tillage systems in areas with poor water availability. In semi-humid areas, rainfall variability leads to annual variation in the biomass production, residue input and soil conditions as a result of the biological transformation of labile organic fractions. We hypothesized that the long-term increase of labile SON fractions is more pronounced under NT than under CT, thus enhancing the N mineralization potential and uptake by wheat crops. However, the differences in these fractions caused by the tillage system depend on a comparison at different soil depths.

This study discusses the long-term effect of two tillage systems on i) the N content in the labile organic matter fractions and their relationship with N mineralization potential at three depths (0–5; 0–10 and 0–20 cm), ii) the factors that affect the N mineralization potential, and iii) the relationship between crop N uptake and N_0 in a semi-humid climate.

2. Materials and methods

2.1. Study site

The study was conducted at the experimental site at Hogar Funke (38° 07′ 06″ S – 62° 02′ 17″ W) in southwestern Buenos Aires province, Argentina (Fig. 1). The soil is classified as a Typic Argiudoll, of over 2 m in depth with a loamy texture in the A horizon and clayey-loamy in the B_2 horizon. According to Thornthwaite, the climate is classified as semi-humid. The rainfall gradient determines an udic soil moisture regime with irregular distribution (Soil Survey Staff, 2010), the rainy seasons being in autumn (March–April) and spring (September–October). The mean annual temperature in this area is 15 °C and the annual precipitation is 735 mm (1887–2012).

A long-term experiment was initiated in 1986 to compare two tillage systems: conventional tillage (CT) and no-tillage (NT). Prior to the establishment of the trial, the site had been cropped under CT for more than 30 years. The CT management was based on two disk operations to mix the residues with the soil: one in the early summer fallow to 15 cm in depth and another before sowing to 10 cm. Meanwhile, NT was characterized by the absence of tillage with over 30% residues covering the soil surface at all times. Under this system, a direct seed drill (John Deere 750 drill, John Deere Argentina S.A.) was used to sow directly into the standing residues of the previous crop. A herbicide ($2\,L\,ha^{-1}$ of glyphosate) was applied for weed control. The plots were fertilized with $10\,kg\,P\,ha^{-1}\,year^{-1}$ as diammonium phosphate (18-46-0) at seeding under both tillage systems. The experiment was designed using a randomized complete block with three replicates. The treatment plot size was $660\,m^2$ ($33\,m \times 20\,m$).

In the years when the plots were sown with winter wheat the soils were sampled at tillering (Z22, Zadoks et al., 1974), as recommended by El-Harris et al. (1983) for studying the N mineralization potential. The full crop sequence and the fallow period differed according to the tillage system and year, as detailed in Table 1. The fallow period was variable for each year and tillage

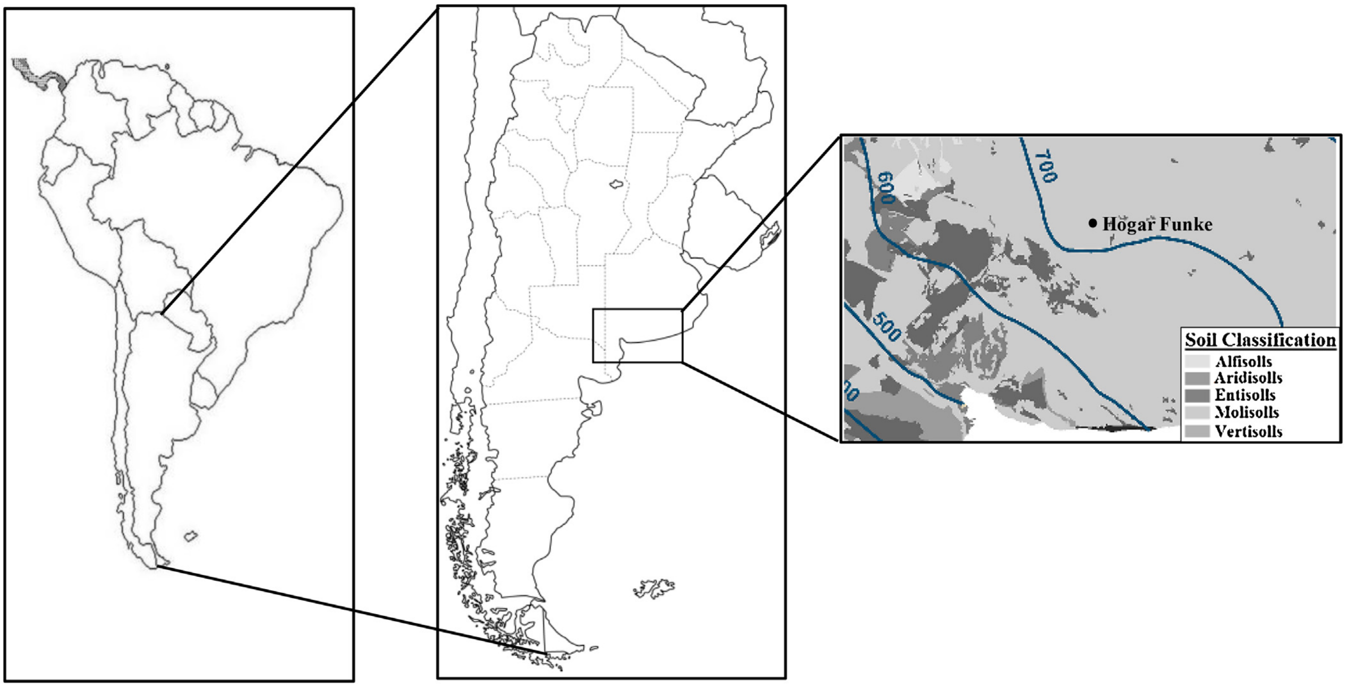

Fig. 1. Location of the long term tillage systems experiment. The selected site is located between the isohyets 700 and 800 mm.

Table 1
Crop sequence, fallow period, wheat yield and aerial residue input by tillage systems in 1986–2011.

Crop Sequence		Fallow period		Crop yield		Aerial residues input	
Year	Crop	CT	NT	CT	NT	CT	NT
		(days)		(kg ha^{-1} year^{-1})			
1986/87	Maize	122	84	3280	3580		
1987	Wheat	64	62	4390	4280	10,975	10,700
1988/89	Sunflower	91	91	890	1080		
1989	Wheat	121	82	4370	4170	10,925	10,425
1990/91	Sunflower	119	93	1400	280		
1991	Wheat	93	36	3260	3050	8150	7625
1992/93	Sorghum	90	70	1510	1550		
1993	Barley	30	25	3070	5000		
1994/95	Maize	62	59	2550	3280		
1995	Barley	121	98	1600	1540		
1996/97	Maize	61	35	1560	3620		
1997	Wheat	30	50	600	1600	1500	4000
1998/99	Maize[a]	176	160	–	–		
1999	Wheat	33	33	1710	2640	4275	6600
2000	Barley	65	85	2884	3000		
2001/02	Sunflower	15	20	1350	1850		
2002	Wheat	35	35	1090	1530	2725	3825
2003	**Wheat**	**54**	**62**	**1769**	**2765**	**4423**	**6913**
2004/05	Sunflower	75	64	2550	2110		
2005	Barley	64	26	1380	1838		
2006/07	Sunflower	215	220	264	214		
2007	Wheat	35	62	1667	2057	4168	5143
2008/09	Maize[a]	190	69	–	–	–	–
2009	**Wheat**[b]	**92**	**210**	**61**	**460**	**153**	**1150**
2010	**Wheat**	**149**	**149**	**1492**	**2538**	**3730**	**6345**
2011	**Wheat**	**124**	**124**	**1049**	**2587**	**2623**	**6468**
Wheat crop	mean	89	79	1951	2516	4877	6290
	SD[c]	52	52	1444	1116	3610	2791
	CV[d]	58	66	74	44	74	44

CT, conventional tillage; NT, no-tillage. Soil sampling years are in bold type. maize (*Zea mays* L.); sunflower (*Helianthus annuus* L.); barley (*Hordeum vulgare* L.); So, sorghum (*Sorghum bicolor* L. Moench).
Bold letters indicate crop in which the sample was taken.

[a] Grazed maize.
[b] Severe drought.
[c] SD, standard deviation.
[d] CV; coefficient of variation.

system: 50–62 days in 2003; 92–210 days in 2009; 149–149 days in 2010, and 124–124 days in 2011 for CT and NT, respectively. The mechanical fallow (CT) was started according to the summer-autumn rainfall occurrence, whereas the chemical fallow (NT) was generally initiated in autumn for weed control. Winter wheat was sown in June/July and harvested by late December. The cultivars and management practices used were those recommended for the region.

The theoretical crop water requirement (CWR) for winter wheat was estimated by the Blaney and Criddle model, adapted to the semiarid region by the Food and Agriculture Organization (FAO) and determined by Paoloni and Vazquez (1985) for the area under study. It was considered equal across all years of the study.

2.2. Soil chemical and physical analyses

Soil sampling was performed over four seasons of the crop sequence (2003, 2009, 2010 and 2011) (Table 1). A composite soil sample was collected at three depths (0–5, 0–10 and 0–20 cm) from each plot of the tillage systems. The soil was air-dried, sieved and homogenized to 2 mm and the retained plant residues were discarded. The soil samples were chemically analyzed to determine: soil organic carbon (SOC) by dry combustion using a Leco C automatic analyzer (Leco Corporation, St Joseph, MI); SON by the micro-Kjeldahl method (Bremner, 1996); pH in a soil-water suspension of 1:2.5; and extractable phosphorus (Pe) (Bray and Kurtz, 1945). The C:N ratio of bulk soil was calculated. Soil organic matter (SOM) was estimated by multiplying the SOC values by the 1.724 factor (Nelson and Sommers, 1982). Available N in the form of nitrate (NO$_3$-N) was determined by steam distillation in soil samples from 0 to 20 and 20–60 cm (Mulvaney, 1996). The SON was assumed to be equal to the total soil N because NH$_4$-N in coarse soils usually accounts for $<0.5\%$ total soil N (Bono and Alvarez, 2013).

The soil particle-size analysis was conducted by the pipette method (Gee and Bauder, 1986). Undisturbed soil samples were also taken at the 0–5, 5–10, 10–15 and 15–20 cm depths with a steel core to determine bulk density (BD) (Blake and Hartge, 1986).

Concentrations were then converted to quantities using BD data. A comparison of the equivalent soil mass between tillage systems was not performed, because previous studies on the same site had found that soil loss was higher in CT than in NT (Galantini et al., 2006). Similarly, Toledo et al. (2013) concluded that the equivalent soil mass method cannot be used to compare tillage systems when erosion has occurred. Table 2 shows details of the site's soil chemical and physical properties at the beginning of this study.

2.3. Potentially mineralizable nitrogen

The N mineralization potential was determined through the anaerobic incubation method developed by Waring and Bremner (1964). A 5 g soil sample was placed in a test tube and 25 mL of distilled water were added. The caps were securely fastened and the tubes were incubated for 7 days at 40 °C under waterlogged conditions. After incubation, the samples were transferred to a distillation flask, rinsed with 25 mL of 4 M KCl and then analyzed for NH_4-N according to Mulvaney (1996). The Nan was calculated by subtracting the quantity of inorganic N -extracted with 2 M KCl in non-incubated samples at room temperature- from the amount in the incubated extract.

The active N fraction of SOM was determined as the ratio between N_0 and SON (N_0/SON*100), as proposed by El Gharous et al. (1990).

2.4. Physical fractionation of SOM by particle size

The physical fractionation of SOM was performed by wet sieving (Cambardella and Elliott, 1992; Duval et al., 2013). Briefly, 50 g of soil previously air-dried and sieved were dispersed in 120 mL glass containers and mixed with 100 mL of distilled water. Ten glass beads (5 mm diameter) were added to increase aggregate destruction and reduce potential problems created by sand (Cambardella and Elliott, 1992). After dispersion, the soil suspension was sieved through two connected sieves (100 μm and 53 μm in diameter) which were moved back and forth. The soil retained in the top sieve was sprinkled with distilled water until the water in the bottom sieve was clear to the naked eye. Two fractions were obtained: i) the coarse particle-size fraction (100–2000 μm) containing coarse sands and coarse particulate organic matter, and ii) the medium particle-size fraction (53-100 μm) containing more stable or fine particulate organic matter, and fine and very fine sand. The fine particle-size fraction (<53 μm) was discarded. In each particle-size fraction, C and N in POM were analyzed for i) particulate organic C and N in the coarse particle-size fraction (cPOM-C and cPOM-N, respectively) and ii) particulate organic C and N in the medium particle-size fraction (fPOM-C and fPOM-N) (53–100 μm). Carbon and N contents in POM were determined using the same methods as for SOC and SON, respectively. The C:N ratio in the particulate organic fractions was calculated for the two fractions, and cPOM-C:N and fPOM-C:N were obtained for the coarse and middle particle-size fractions, respectively.

2.5. Crop nitrogen uptake

At physiological maturity (Z90), the above-ground biomass of wheat was harvested manually from two 0.25 m^2 areas per plot. The dry matter was determined after drying in a forced-air oven at 60 °C for at least 72 h. The grain was separated from the straw and they were both weighed. The N concentration in the total aerial biomass (grain and straw) was determined by the standard micro-Kjeldahl method (Bremner, 1996). The total aerial residue input (kg ha^{-1}) was calculated with the residue input from the previous crop for each studied year. On the basis of the grain yields from previous crops in the sampling years, the aerial biomass of the residue was estimated considering a harvest index of 0.40 for wheat (Studdert and Echeverría, 2000; Wyngaard et al., 2012).

2.6. Statistical analysis

All data in the tables and figures are presented as means. Differences in the results due to the treatment were tested by a two-way ANOVA, considering the year and tillage system as fixed effects. The means of the main effects were compared using the least significant difference test when differences between treatment means were significant ($P < 0.05$). A Pearson's correlation analysis was performed to assess the relationship of N_0 with soil C and N organic fractions, available N, the fallow period, residue input and wheat N uptake. The statistical analysis was carried out with Infostat software (Di Rienzo et al., 2013).

3. Results

3.1. Weather, wheat production and crop N uptake

Rainfall was concentrated in the periods from January to March and from September to December, though it varied over the years (Fig. 2). In 2003, a water deficit was observed in September and November, and an excess in October. In 2009, winter rainfall was exceptionally low, thus hindering the normal development of winter wheat. In 2009 and 2010, water shortages occurred in October. In 2011, there was a sharp deficit in early spring and a water excess in November. In general, the monthly rainfall was lower than the historical records for April–May, i.e. the months of wheat fallow. There was great variability over the years during the period that determines wheat yield, i.e. September-October-November, under semi-humid conditions. Rainfall during the fallow period and at crop sowing was above the CWR in 2003, 2010 and 2011.

Wheat total aerial biomass and grain yields were consistently higher under NT than under CT over the four years (Fig. 3) ($P < 0.01$). On average, wheat grain yields from 1986 to 2011 were

Table 2
Chemical and physical properties of soil at the beginning of the study (year 2003).

Depth (cm)	Tillage system	SOM	SON	Pe	pH	BD	Texture		
							Sand	Silt	clay
		(g kg^{-1})		(mg kg^{-1})		(Mg m^{-3})	(g kg^{-1})		
0–20	NT	31	1.49	17	6.5	1.39	321	426	253
	CT	27	1.35	15	6.7	1.30	338	422	240

CT, conventional tillage; NT, no-tillage; SOM, soil organic matter (g kg^{-1} soil); SON, soil organic nitrogen (g kg^{-1}); Pe, extractable phosphorus (mg kg^{-1}); BD, bulk density (Mg m^{-3}).

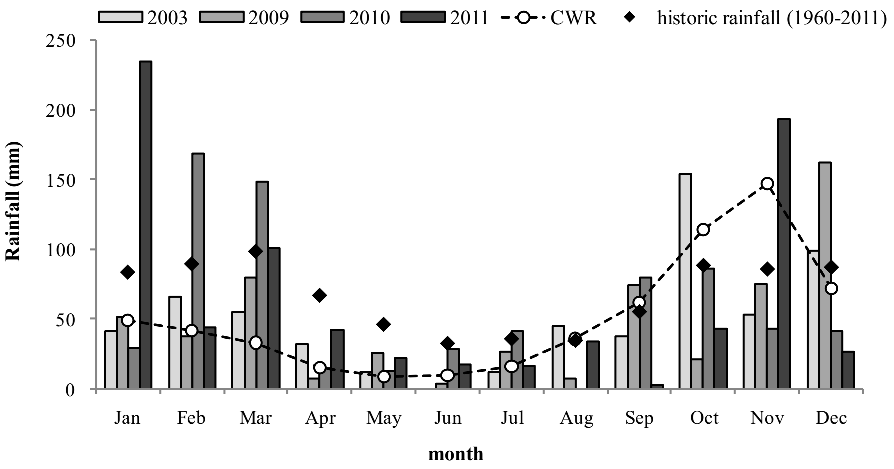

Fig. 2. Monthly rainfall over the sampled years, crop water requirement (CWR) and historic rainfall during 1960–2011.

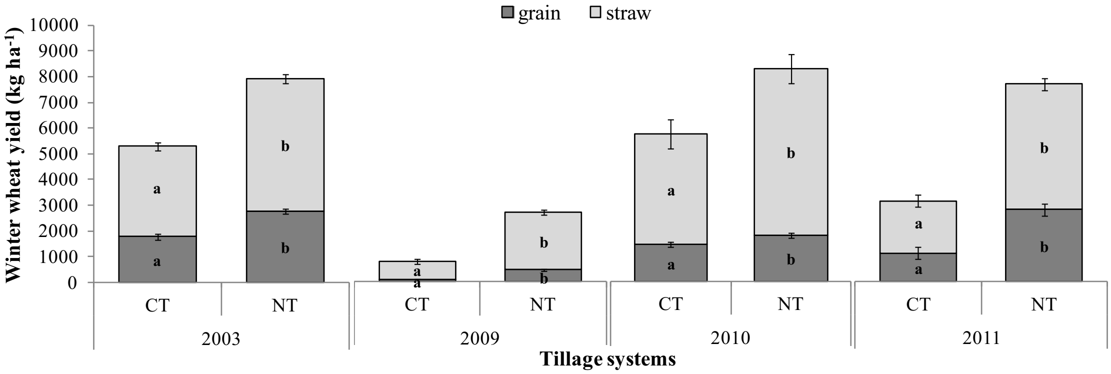

Fig. 3. Grain and total aerial biomass (grain and straw) yield by tillage systems: no-tillage (NT) and conventional tillage (CT) and years. For each year different letters indicate statistically significant differences between tillage systems (P < 0.01).

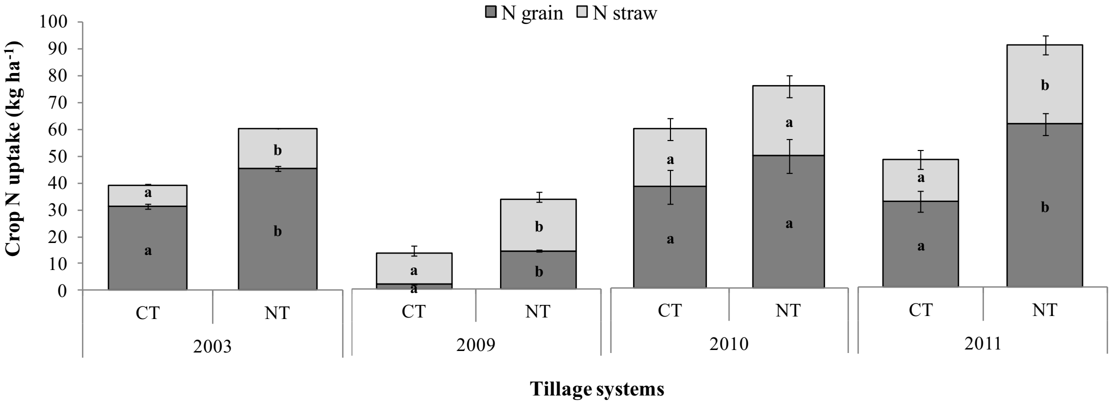

Fig. 4. Nitrogen uptake by wheat by tillage systems: no-tillage (NT) and conventional tillage (CT) and years. For each year different letters indicate statistically significant differences between tillage systems (P < 0.001).

29% higher in NT and showed greater stability, as indicated by the lower coefficient of variation (CV) (Table 1).

Crop N uptake showed significant interactions between treatments and years ($P < 0.001$), so the treatment effect was analyzed separately in each sampling year (Fig. 4). Highly significant differences ($P < 0.001$) in N uptake by grain and straw were observed as a result of the tillage system in 2003, 2009 and 2011. However, no significant differences were found in 2010.

Regarding the relationship of total aerial biomass and grain yield with crop N uptake by tillage system for 2003, 2010 and 2011 (data for 2009 were excluded from the analysis because of the severe drought that affected wheat performance), no significant relationships ($P > 0.05$) were found between wheat parameters for CT and NT (data not shown).

3.2. Soil organic nitrogen, nitrogen in soil organic fractions, potentially mineralizable nitrogen and active nitrogen fraction

The SON (Mg N ha^{-1}) showed highly significant differences ($P < 0.001$) between tillage systems and years at each depth (0–5, 0–10 and 0–20 cm) (Table 3); a significant interaction was detected between treatments and years, so the treatments were analyzed separately by depth and year. At the 0–5 cm depth, SON was significantly different ($P < 0.001$) between the tillage treatments in 2003, 2010 and 2011, but these differences were not detected in 2009 ($P > 0.05$). Estimates of SON under NT were 20 to 35% greater than under CT. Similar patterns were found at 0–10 cm and 0–20 cm, but to a lesser degree.

The average content of cPOM-N and fPOM-N was higher in NT for all depths and years (Table 3), with significant differences ($P < 0.01$) between tillage systems in 2003, 2009 and 2010. However, in 2009 this difference was not detected for cPOM-N at the 0–20 cm depth and in 2011 it was similar to this finding at the three depths. In 2009, N-content in soil fractions declined more sharply in the topsoil (0–5 and 0–10 cm) under NT.

The N_0 stock was higher ($P < 0.001$) under NT than under CT across the years of the study in the 0–5 cm soil layer (Table 3), except for 2009. The average values were 47% higher under NT as compared to CT at 0–5 cm. Significant differences in N_0 were found between the years, where 2003 > 2011 > 2009 = 2010 at 0–5 cm.

Table 3

Soil organic nitrogen, particulate organic matter nitrogen and potentially mineralizable N (mean ± standard deviation) and variance analysis by tillage system and years at each depth.

Soil organic N fraction	Tillage System	Year	Depth (cm)		
			0–5	0–10	0–20
SON			(Mg N ha^{-1})		
	NT	2003	1.27 ± 0.05	2.32 ± 0.07	4.15 ± 0.09
		2009	0.93 ± 0.14	1.76 ± 0.09	3.33 ± 0.06
		2010	1.04 ± 0.15	1.95 ± 0.18	3.58 ± 0.24
		2011	0.92 ± 0.06	2.01 ± 0.10	3.98 ± 0.22
	CT	2003	0.81 ± 0.09	1.75 ± 0.16	3.50 ± 0.09
		2009	0.79 ± 0.03	1.72 ± 0.01	3.41 ± 0.20
		2010	0.72 ± 0.04	1.49 ± 0.02	2.94 ± 0.10
		2011	0.72 ± 0.04	1.52 ± 0.05	3.19 ± 0.02
	Tillage system (TS)		***	***	***
	Year (Y)		***	***	***
	TSxY		***	**	***
POM			(Mg POM-N ha^{-1})		
cPOM-N	NT	2003	0.082 ± 0.006	0.126 ± 0.011	0.181 ± 0.003
		2009	0.101 ± 0.007	0.139 ± 0.008	0.169 ± 0.011
		2010	0.095 ± 0.007	0.132 ± 0.010	0.167 ± 0.012
		2011	0.076 ± 0.006	0.107 ± 0.008	0.138 ± 0.009
	CT	2003	0.050 ± 0.002	0.092 ± 0.004	0.124 ± 0.006
		2009	0.050 ± 0.005	0.107 ± 0.009	0.159 ± 0.011
		2010	0.039 ± 0.003	0.087 ± 0.004	0.130 ± 0.006
		2011	0.067 ± 0.003	0.125 ± 0.006	0.157 ± 0.010
	Tillage system (TS)		**	**	**
	Year (Y)		**	***	*
	TSxY		**	***	**
fPOM-N	NT	2003	0.126 ± 0.011	0.299 ± 0.012	0.606 ± 0.015
		2009	0.252 ± 0.025	0.507 ± 0.029	0.810 ± 0.033
		2010	0.271 ± 0.022	0.501 ± 0.026	1.001 ± 0.031
		2011	0.164 ± 0.016	0.320 ± 0.019	0.693 ± 0.026
	CT	2003	0.066 ± 0.004	0.181 ± 0.006	0.504 ± 0.012
		2009	0.150 ± 0.006	0.328 ± 0.008	0.600 ± 0.016
		2010	0.167 ± 0.009	0.370 ± 0.015	0.754 ± 0.023
		2011	0.100 ± 0.004	0.226 ± 0.008	0.410 ± 0.009
	Tillage system (TS)		***	**	*
	Year (Y)		**	**	**
	TSxY		*ns*	*ns*	*ns*
N_0			(kg N_0 ha^{-1})		
	NT	2003	64.1 ± 4.1	106 ± 8.1	180 ± 12
		2009	32.4 ± 1.8	59.7 ± 4.6	90.8 ± 22
		2010	29.1 ± 1.2	51.2 ± 3.3	108 ± 32
		2011	44.8 ± 2.8	75.6 ± 5.5	113 ± 9.3
	CT	2003	39.6 ± 4.6	78.1 ± 4.0	130 ± 4.8
		2009	24.4 ± 5.0	46.0 ± 5.8	77.0 ± 2.6
		2010	22.6 ± 1.2	41.9 ± 1.8	83.3 ± 8.4
		2011	29.5 ± 6.8	53.5 ± 9.5	75.0 ± 10
	Tillage system (TS)		***	***	***
	Year (Y)		***	***	***
	TSxY		***	*ns*	*ns*

CT, Conventional tillage; NT, no-tillage. SON, soil organic nitrogen; cPOM-N, coarse particulate organic nitrogen; fPOM-N, fine particulate organic nitrogen. N_0: potentially mineralizable N. *, **, ***, significant differences at 0.05; 0.01, 0.001 probability levels, respectively; ^{ns}, not significant.

Table 4
Active N fraction (mean ± standard deviation) and variance analysis by tillage systems and years at each depth.

Tillage system	Years	Depth (cm)		
		0–5	0–10	0–20
		(%)		
NT	2003	5.1 ± 0.5	4.6 ± 0.4	4.3 ± 0.4
	2009	3.5 ± 0.4	3.4 ± 0.1	2.7 ± 0.7
	2010	2.8 ± 0.5	2.7 ± 0.4	3.0 ± 0.7
	2011	4.9 ± 0.5	3.8 ± 0.3	2.8 ± 0.2
CT	2003	4.9 ± 0.8	4.5 ± 0.3	3.7 ± 0.2
	2009	3.1 ± 0.5	2.7 ± 0.3	2.3 ± 0.2
	2010	3.2 ± 0.1	2.8 ± 0.1	2.8 ± 0.4
	2011	4.1 ± 0.8	3.5 ± 0.7	2.4 ± 0.3
Tillage system (TS)		*ns*	*ns*	***
Year (Y)		***	***	***
TS × Y		*ns*	*ns*	*ns*

CT, Conventional tillage; NT, no-tillage. Active N fraction, N_0/SON. *, **, ***, significant differences at 0.05; 0.01, 0.001 probability levels, respectively; ^{ns}, not significant.

Also, significant differences ($P < 0.001$) between tillage systems and years were observed for the 0–10 cm depth, with no evidence of interaction. Similar significant differences were found in N_0 between tillage systems ($P < 0.001$) at the 0–20 cm depth.

The proportion of SON that represents the N_0 –referred to as the active N fraction of SOM- showed highly significant differences ($P < 0.001$) over the years for both tillage systems (Table 4). However, significant differences between the tillage systems were found only at 0–20 cm, with higher values under NT.

3.3. Relationship between potentially mineralizable N and different variables

On assessing the relationships between the N_0 and SOM fractions, high correlations were observed for both tillage systems (Table 5). Significant ($P < 0.05$) and positive correlations between N_0 and fPOM-C were found at the three depths, whereas highly significant ($P < 0.001$) and negative relationships between N_0 and fPOM-N were found under both tillage systems at 0–5 and 0–10 cm. Also, positive correlations were detected between N_0 and cPOM-C at all three depths, and a negative relationship between N_0 and cPOM-N at 0–20 cm under CT (Table 5). For both tillage systems and each depth, highly significant and positive correlations ($P < 0.001$) were found between N_0 and the fPOM-C:N ratio, except in CT at 0–20 cm. In addition, no correlations were observed between N_0 and the C:N ratio for both tillage systems and for each depth. Highly significant ($P < 0.01$) and negative correlations occurred between N_0 and available N under both tillage systems and for each depth. Significant and negative correlations ($P < 0.05$) were found between N_0 and the fallow period by tillage system at each depth. In addition, a significant effect of residue input from previous crops was observed on N_0 under both tillage systems and for all three depths. The relationship between N_0 and crop residues decreased with depth under NT, the peak being at 0–5 cm, whereas higher correlations were found in the first 10 cm of soil under CT.

No significant correlations were found for any tillage system or depth between N_0 and wheat N uptake by tillage system at each depth.

4. Discussion

4.1. Crop production and N uptake

Higher yields under NT and a greater stability (lower CV in wheat crops) were in agreement with Govaerts et al. (2006), who compared two tillage systems in a long-term experiment in the semi-arid region of Mexico. These differences between tillage systems may be attributed to a more favorable water balance under NT as a result of lower evaporation rates in a semi-humid climate, which in turn leads to higher yields (Buschiazzo et al., 1998; Bono et al., 2008). In contrast, under no water-limiting conditions, Fabrizzi et al. (2005) found similar wheat yields in Mollisols for CT and NT. The high annual variation in wheat yields showed the effect of irregular rainfall on wheat performance and the subsequent variation of residue input to the soil over the years (Table 1).

When year 2009 was excluded from the analysis, no relationships were detected between the total aerial biomass, grain yield and crop N uptake at Z90. In this region, N accumulation in wheat does not result in higher grain yields because of adverse weather

Table 5
Pearson's correlations between potentially mineralizable N (N_0) and soil organic nitrogen fractions, C:N ratios, available N, fallow period and residue input by tillage system and depth.

Depth (cm)	Tillage system	cPOM-N	fPOM-N	cPOM-C	fPOM-C	SOC	SON	C:N	cPOM-C:N	fPOM-C:N	Available N	Fallow period	Residue input	Crop N uptake
		Pearson correlation coefficients (r) with N_0												
0–5	NT	−0.69	−0.94	−0.09	0.90	0.46	0.61	−0.44	0.14	0.98	−0.85	−0.88	0.75	−0.03
	(P-value)	*	***	*ns*	***	*ns*	*	*ns*	*ns*	***	***	***	**	*ns*
	CT	0.25	−0.83	0.75	0.67	0.73	0.47	0.49	0.30	0.85	−0.77	−0.69	0.79	−0.03
	(P-value)	*ns*	***	**	*	**	*ns*	*ns*	*ns*	***	**	*	**	*ns*
0–10	NT	−0.05	−0.86	0.15	0.79	0.61	0.77	−0.45	−0.16	0.88	−0.83	−0.85	0.70	−0.09
	(P-value)	*ns*	***	*ns*	**	*	**	*ns*	*ns*	***	***	***	*	*ns*
	CT	−0.13	−0.85	0.72	0.66	0.69	0.49	0.45	0.52	0.94	−0.88	−0.79	0.84	−0.06
	(P-value)	*ns*	***	**	*	*	*ns*	*ns*	*ns*	***	***	**	***	*ns*
0–20	NT	0.43	−0.60	0.16	0.68	0.50	0.77	−0.51	0.07	0.76	−0.88	−0.66	0.66	−0.04
	(P-value)	*ns*	*	*ns*	*	*ns*	**	*ns*	*ns*	**	***	**	*	*ns*
	CT	−0.75	−0.15	0.65	0.66	0.43	0.45	−0.16	0.72	0.50	−0.92	−0.74	0.72	0.03
	(P-value)	**	*ns*	*	*	*ns*	*ns*	*ns*	**	*ns*	***	**	**	*ns*

NT, no-tillage; CT, conventional tillage; N_0, potentially mineralizable N ($kg\,ha^{-1}$); cPOM-N, nitrogen in coarse particulate organic matter ($Mg\,ha^{-1}$); fPOM-N, nitrogen in fine particulate organic matter ($Mg\,ha^{-1}$); cPOM-C, coarse particulate organic carbon ($Mg\,ha^{-1}$); fPOM-C, fine particulate organic carbon ($Mg\,ha^{-1}$); SOC, total organic carbon ($Mg\,ha^{-1}$); SON, total organic nitrogen ($Mg\,ha^{-1}$); C:N, carbon- nitrogen ratio in whole soil; cPOM-C:N, carbon- nitrogen ratio in the coarse particulate fraction; fPOM-C:N, carbon- nitrogen ratio in the fine particulate fraction; available N, inorganic N in nitrates form ($kg\,ha^{-1}$) at sowing at 0–60 cm. *, **, ***, significant correlation at 0.05; 0.01, 0.001 probability levels, respectively; ^{ns}, not significant.

conditions during the critical period of the wheat crop, i.e. during remobilization of photo-assimilates to the grain (Martínez et al., 2015). This result would indicate that under these variable weather conditions crop N uptake can be used to assess N mineralization more accurately than total aerial biomass or grain yield. In addition, Sahrawat (1983) reported that N uptake by plants is a better criterion for studying the soil N-availability than total biomass and grain yield.

4.2. Soil organic nitrogen, potentially mineralizable nitrogen and particulate organic matter

The results showed that values of soil labile N fractions and N_0 were higher under NT in the long term, as hypothesized. These higher values, however, were found at all depths (0–5; 0–10 and 0–20 cm) and not only in the most superficial layer. The results suggest that these soils in particular fail to show differences in the effect of tillage on labile N fractions according to the sampling depth. One possible explanation of this result could be the degradation of the soil by erosion. Although not discussed in this paper, the important benefit of NT in reducing soil erosion compared with CT needs to be considered in any comparison of tillage systems for semiarid climates (McConkey et al., 1996). Furthermore, Galantini et al. (2006) reported soil loss by erosion under CT when working at the same site of the present study, which may explain these differences between SON and labile fractions according to the tillage system at all depths. Similar results were observed by Fabrizzi et al. (2003), who found a higher content of SON and labile fractions at various depths under contrasting tillage systems in Mollisols; they attributed these results to soil degradation caused mainly by erosion after several years under continuous grain cropping, a condition comparable to this study.

Potentially mineralizable N showed trends that were similar to SON and particulate organic N fractions by tillage system and depth, which would suggest that it could be highly dependent on the labile fractions of N, as reported by other authors (Balesdent et al., 2000; Mikha et al., 2006; Sharifi et al., 2008). Also, significant differences were found across the sampling years according to the tillage system for the three depths evaluated, however, significant interactions were found between the tillage system and year at 0–5 cm. This differential effect on the most superficial layer according to the tillage system may be due to the differential residue contribution by years (Table 1), which depends on variability in the climatic conditions. As was mentioned by several authors (Fabrizzi et al., 2003; Cozzoli et al., 2010; Reussi Calvo et al., 2013), N_0 is closely related to the weather conditions. Despite the higher content of SON fractions under NT, the active N fraction of SOM only showed significant differences between tillage systems at the 0–20 cm depth, with higher values under NT. This would indicate that long-term tillage systems under semi-humid conditions may affect SOM quality only at 0–20 cm. However, this result may not be due to an increase in labile N fractions at this depth but rather to a greater protection of these fractions in the undisturbed soil (Beare et al., 1994; Six et al., 1999). Staley et al. (1988) found that when NT is imposed on a soil previously under CT, the trend would be towards the rebuilding of an active soil N-pool with a slower turnover rate, which is continually mineralized under CT. It is important to emphasize that this fraction is considered as the portion of SOM that supplies the highest plant-available N for crop growth, as proposed by El Gharous et al. (1990).

4.3. Relationship between potentially mineralizable nitrogen and the variables analyzed

In most cases, the highly positive correlations of N_0 with fPOM-C and negative with fPOM-N can be explained by the fPOM-C:N ratio, i.e. by the quality of this labile fraction. The fPOM-C:N ratio ranged from 6 to 21 (data not shown) and, according to Haynes (2005), the C:N ratio should be under 25 for N to be mineralized. When the C:N ratio is below that threshold, microorganisms do not have enough energy and they release mineralized N which exceeds immobilization (Jones and Parsons, 1970). This is why the C:N ratio would determine if microorganisms are C- or N-limited (Bengtsson et al., 2003). In this case, these results may indicate a C limitation under both tillage systems, because POM-C is a substrate for the heterotrophic microorganisms responsible for N mineralization (Divito et al., 2011). It is worth noting, however, that labile C comes from POM, and the residues input that regulates POM is highly variable in this environment because of the climatic conditions. The correlation of N_0 with the fPOM-C:N ratio decreased with the sampling depth under NT, thus indicating stratification of the more labile fractions. In contrast, equal relationships between N_0 and fPOM-C:N were observed at 0–5 and 0–10 cm under CT because the tillage depth was 15 cm. This would also explain the high correlation between N_0 and cPOM-C at 0–10 cm under CT as a result of a more homogeneous distribution of residues at depth, i.e. POM, which is more susceptible to degradation by microorganisms (Balesdent et al., 2000; Villamil et al., 2015). These results showed the close relationship between N_0 and labile organic C fraction under CT and NT, thus indicating that N_0 is regulated by these fractions regardless of the tillage system. Under NT, the N_0 was mainly regulated by fPOM-C and its quality, whereas under CT it was influenced by cPOM-C, fPOM-C and its quality. It is important to emphasize that these organic fractions rely on the crop residue input (Duval et al., 2016).

Residues have a positive impact on labile organic fractions (Galantini et al., 2006), thereby modifying the part of SOM that supplies the greatest portion of plant-available N. In this case, the effect of residues could be confirmed by the high and positive correlation between N_0 and the residue input from the preceding crop (Table 5). On the other hand, the differences between these fractions found at depth under both tillage systems would be associated with the amount and location of crop residues, i.e. on the soil surface under NT and incorporated and homogenized at 10–15 cm under CT. These results show that the fallow period and residue input affect N_0 differently, regardless of the tillage system. This inverse relationship would be due to the climatic conditions, especially water availability from rainfall during fallow, when mineralization is increased and N is converted into available forms (Studdert and Echeverría, 2000; Galantini and Rosell, 2006), whereas the amount of residues favors the availability of energy substrate for the microorganisms (Divito et al., 2011). However, these relationships would result from the close correlations between labile the C and N fractions (fPOM-C and fPOM-N) with the fallow period and the residue input (data not shown), which are highly related to N_0, as previously mentioned. On the other hand, Galantini and Rosell (2006) reported the effect of fallow and growing season rainfalls on the dynamics of labile SOM under these rainfed production systems.

No significant correlations between N_0 and wheat N uptake ($P > 0.05$) were found for any tillage system or depth. This result did not confirm the hypothesis that greater N_0 results in higher N availability and N uptake by wheat, despite the higher values of all labile N fractions under NT. These findings differed substantially from those of Chalk and Waring (1970), who reported high and positive relationships between N_0 and wheat N uptake in a pot experiment under conditions of controlled water availability. However, Christensen and Mellbye (2006) found poor relationships between N_0 determined by anaerobic incubation and wheat N uptake under both CT and NT, as was confirmed by the present study. For this reason, the impact of a higher potential mineralization under NT on the increase in N availability and N uptake by crop cannot be verified under these semi-humid conditions because of the water deficits during the growing season (Fig. 2). Therefore, N_0 determined by anaerobic incubation could be used as an indicator of potential N-availability under these conditions, regardless of the tillage system.

This study helps to better understand the complex dynamics of SON for different tillage systems in a semi-humid climate. Under these specific conditions, the N balance may vary during the growing season as a result of the interaction between the labile organic fractions and water availability. The labile pools of SON should hence be studied in further detail over time, i.e. weekly or monthly.

5. Conclusions

The greater content of SON fractions under NT was associated with a higher mineralization potential, however, it did not result in increased N availability and N uptake by wheat, because of climatic conditions during the crop growing season.

Soil organic N fractions were shown to be strongly influenced by residue input from the previous crop and by variable weather conditions during the fallow period. The N_0 proved to be positively influenced by the quantity and quality of POM-C regardless of the tillage system.

For further research, it would be essential to understand the factors affecting the turnover of N_0 and release of available N to crops during the growing season in a semi-humid climate.

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