

**Ammonium accumulation in soil: the long-term effects of tillage,
rotation and N rate in a Mediterranean Vertisol**

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Running title: Ammonium accumulation in a Vertisol

Summary

Plant nutrition requires organic nitrogen to be mineralised before roots can absorb it. A 13-year field study was conducted on typical rain-fed Mediterranean Vertisol to determine the effects of tillage system, crop rotation and N fertiliser rate on the long-term $\text{NH}_4^+\text{-N}$ content in the soil profile (0–90 cm). The experiment was designed as a randomised complete block with a split-split plot arrangement and three replications. The main plots tested the effects from the tillage system (no-tillage and conventional tillage); the subplots tested crop rotation with two-year rotations (wheat-wheat, wheat-fallow, wheat-chickpea, wheat-faba bean and wheat-sunflower) and the sub-subplots examined the N fertiliser rate (0, 50, 100 and 150 kg N ha⁻¹). Soil $\text{NH}_4^+\text{-N}$ content was greatest in the rainiest years and greater under the no-tillage (NT) system than the conventional tillage (CT) system (57 and 48 kg ha⁻¹, respectively). The deepest soil (30-60 cm and 60-90 cm) contained a greater $\text{NH}_4^+\text{-N}$ content (21.0 and 21.4 kg ha⁻¹, respectively) than the shallowest soil (19.5 kg ha⁻¹ in 0-30 cm). This observation may be related to Vertisol characteristics, especially crack formation that allows greater mineralization in the deepest layers by displacing organic matter.

Keywords: no-tillage, wheat, faba-bean, chickpea, sunflower, rainfed

Introduction

The concept of mineralisation is not used consistently by authors. Certain authors define mineralisation as the transformation of organic N to an inorganic form (e.g., $\text{NH}_4^+\text{-N}$, NH_3 and $\text{NO}_3^-\text{-N}$) (e.g. Johnston & Jenkinson, 1989; Van den Bossche et al., 2009). However, other authors consider mineralisation synonymous with ammonification

(transformation of organic N to $\text{NH}_4^+\text{-N}$ and NH_3) (e.g. Jarvis et al., 1996; Kumar & Goh, 2000; Martens, 2001). Mineralisation is an important process in plant nutrition because it is generally accepted that release of a nutrient from organic form is required before plant roots can absorb it. (Marschner, 1986). Mineralisation is the heterotrophic microbial transformation of N from an organic state to inorganic NH_4^+ or NH_3 (Loomis & Connor, 1992; Gerik et al., 1998; Kumar & Goh, 2000; Martens, 2001). Mineralisation is almost always coupled with immobilisation, and the two processes are intimately connected and dependent. Immobilised N is likely subsequently available for mineralisation when the microbial population turns over (Jarvis et al., 1996). According to Loomis & Connor (1992), ammonification primarily depends on aerobic bacteria, and a neutral pH, moisture, good aeration and an appropriate carbon substrate facilitate this process. Additionally, nitrogen fertiliser can have a preparatory effect on ammonification by stimulating bacterial growth.

Crop rotations can have different impacts on mineralizable N pools and N availability through the quantity and quality of added residues (Carpenter-Boggs et al., 2000; Senwo and Tabatabai, 2005). Bedard-Haughn et al. (2013) observed no difference in gross mineralisation among rotations with or without pulses. Sharifi et al. (2008) did not observe greater mineralizable N in rotation with legumes. However, Carpenter-Boggs et al. (2000) reported that rotation with legumes increased the N mineralizable from soil to support crop production.

Several authors disagree on the influence of tillage systems on mineralisation. Jarvis et al. (1996) and Dong et al. (2012) reported that ploughing caused more mineralisation than establishing crops through direct drilling. However, Muruganandam et al. (2010) reported greater N mineralisation rates for NT than CT (no-tillage and conventional tillage, respectively) management. To different degrees, mineralisation occurs for both

new and existing residues as well as degraded organic material at varying ages and recalcitrance levels (Jarvis et al., 1996).

Vertisols are fine soils with swelling clay minerals that develop wide and deep cracks during prolonged dry seasons. When dry, such soils have a hard consistency but they are plastic and sticky when wet (Lal, 1989). Vertisols pose specific tillage problems and have particular requirements; their properties vary considerably depending on location and timing. Among other things, conventional and continuous cultivation increases bulk density and soil strength and degrades the macropore space and pedality. In contrast, minimum or zero tillage systems and stubble retention can improve soil structure, increase organic matter levels (Blair & Crocker, 2000) as well as soil water storage capacity (O'Leary & Connor, 1997), improve chemical fertility (Chan et al., 1999) and conserve water (Carroll et al., 1997).

Feigin & Yaalon (1974) and Chen et al. (1989) reported ammonium retention in Vertisols. Clay minerals with a large layer charge may strongly retain ammonium. Ammonium retention is not the only way to reduce its availability to plants. Other pathways include loss through ammonia volatilisation, nitrate leaching after bacterial transformation and loss through denitrification (Coulombe et al., 1996).

According to Böckman et al. (1990), ammonium in the soil is due to the following: i) organic nitrogen mineralisation in manure, plant and humus residue; ii) ammonium fertiliser application; iii) aerial deposition; or iv) fertiliser urea hydrolysis, which is rapid process and completed a few days after application. Positively charged ammonium can be absorbed by soil colloids, which are present at small levels in a soil solution. In general, when NH_4^+ quantity declines in a soil solution, clay particles release more NH_4^+ into the soil solution (Gerik et al., 1998). In certain soils, NH_4^+ may be bound so tightly to clay particles that it cannot be readily released into the soil solution for plant uptake; NH_4^+ is

‘fixed’ or ‘nonexchangeable’ under such conditions. In addition to direct uptake through interception, NH_4^+ ion movement toward the root surface area primarily depends on diffusion, NH_4^+ diffuses around a point with a ball-like shape, and its concentration is inversely proportional to the diffusion distance (Li et al., 2013). According to Xuan (1985) and Sun (1987), if the soil water content approaches saturation, and the water includes high NH_4^+ ion levels after N fertilizer application in particular, NH_4^+ -N may penetrate deeply into a low soil layer through mass flow.

Numerous studies have investigated mineralisation defined as the process for transforming organic N to inorganic N (mineralisation rate and total mineral N, etc.). However, few studies have investigated ammonium quantity in the soil. Defining mineralisation as transformation of organic N to NH_4^+ -N, the objective for this study was to determine the influence of the tillage system, crop rotation and N rate on NH_4^+ -N accumulation in a Vertisol profile under Mediterranean dryland conditions over 13 years of a long-term experiment.

Materials and methods

Site and experimental design

Field experiments were conducted in Córdoba, southern Spain (37° 46' N and 4° 31' W, 280 m a.s.l.), on a Vertisol (Typic Haploxererts) typical of the Mediterranean region, where rainfed cropping is the standard practice (Table 1). The study took place over a 13-year period within the framework of a long-term experiment called “Malagón” that began in 1986 and was designed as a randomised complete block with a split-split plot arrangement and three replications. The main plots tested the effects of the tillage system (no-tillage and conventional tillage); subplots tested two-year crop rotations (wheat-

wheat, wheat-fallow, wheat-chickpea, wheat-faba bean and wheat-sunflower); and sub-subplots tested N fertiliser rate (0, 50, 100 and 150 kg N ha⁻¹). Each rotation was duplicated in a reverse crop sequence to obtain data for all crops on a yearly basis. The area of each sub-subplot was 50 m² (10 by 5 m).

Crop management

The no-tillage plots were seeded with a no-till seed drill. Weeds were controlled with glyphosate [N-(phosphonomethyl) glycine] + MCPA [(4-chloro-2-methylphenoxy) acetic acid] at a rate of 0.5 + 0.5 L a.i. ha⁻¹ prior to planting. The conventional tillage treatment included mouldboard ploughing and disk harrowing or vibrating tine cultivation to prepare a suitable seedbed. During the chickpea and faba bean growing season, weeds were controlled by means of cyanazine [2-(4-chloro-6-ethylamino-1,3,5-triazin-2-yl-amino)-2-methyl propionitrile] at 2 L a.i. ha⁻¹. Ascochyta blight (*Didymella rabiei*) in chickpea was controlled with chlorothalonil [2, 4, 5, 6 – tetra-chloroisophthalonitrile] at 0.75 a.i ha⁻¹. Glyphosate was applied to faba bean plots at rate of 0.065 L a.i. ha⁻¹ as a post-emergence spray when broomrape (*Orobanche crenata* Forsk) was approximately 0.5–1 cm high (García-Torres et al., 1987).

As is common practice in the area, kabuli chickpea (cvs Pedrosillano and Zoco) was planted in 0.38-m-wide rows in February at a seeding rate of 80 kg ha⁻¹; faba bean (cv Alameda) was planted in 50-cm-wide rows in early December at 170 kg ha⁻¹; sunflower (various hybrid cultivars) was planted in 50-cm-wide rows in February at 5 kg ha⁻¹; and hard red spring wheat (cvs Cajeme and Gazul) was planted in 18-cm-wide rows in late November at 150 kg ha⁻¹. Each year, the wheat plots were also supplied with P fertiliser at a rate of 65 kg P ha⁻¹; the fertiliser was incorporated following the standard practice in

the conventional tillage soil and was banded with drilling in the no-tillage plots. Available P at the end of the experiment was greater than at the beginning for all depths due to the systematic supply of fertiliser described above (Table 1).

The wheat grain yield (harvested in early June) was determined using a 1.5m wide Nurserymaster Elite Plot Combine (Wintersteiger, Ried, Austria) (30m² per plot). Nitrogen uptake was determined by the analysis of N content of straw and grain, using the Dumas combustion method (Leco FP-428 analyzer).

Soil analysis

Three soil core samples were collected from each wheat plot prior to sowing 0–90 cm deep using a manual Eijkelkamp auger. Every three years, samples were divided into three portions: 0 to 30, 30 to 60 and 60 to 90 cm to analyze each soil depth. Due to the soil's high clay levels, the samples were dried before grinding and sieving. The samples were crumbled as finely as possible and spread on trays to facilitate air-drying for 48 h. Subsequently, they were ground, sieved (2 mm) and maintained (frozen) in plastic bags at –30°C until analysis. The content of soil ammonium was determined using a colorimetric method (Griess Illosvay modified by Barnes & Folkard, 1951 and Bremner, 1965) with a continuous flow colorimeter (QUAATRO, Bran Luebbe, Norderstedt, Germany).

A Delta-T meteorological station in the study area was used. It included a soil temperature probe to measure soil temperature hourly at 20 cm depth.

Statistical analysis

We analyzed the data using a mixed-model analysis of variance (ANOVA) with three fixed factors: tillage system, crop rotation and N rate. The year was a random factor due to unpredictable weather conditions in the rain-fed Mediterranean environment (Gómez & Gómez, 1984). The ammonium content were analyzed through ANOVA using a split-split plot design for the years investigated, tillage system, preceding crop and N rate following the error method reported by McIntosh (1983). Where the effects were significant, the treatment methods were compared using Fisher's protected least significant difference (LSD) test with the significance threshold $P = 0.05$. The ANOVA analyses were performed using Statistix 8.1 (Analytical Software, 2005) to determine the treatment effects.

Results

Weather conditions

The years with the most annual precipitation were 1996 and 1997 (953 and 935 mm, respectively), and the years with small annual precipitation were 2005, 1999 and 1995 (263, 281 and 284 mm, respectively) (Figure 1). The seasonal rainfall distribution differed among the years studied. The rain was primarily concentrated in autumn; the average precipitation in autumn during the study period was 250 mm; it was 44, 108 and 171 mm in summer, spring and winter, respectively (Fig. 1 and 2).

The average soil temperatures in summer were greatest in 2001 and 2002 (30.8 and 30.1 °C, respectively) (Fig. 1). Conversely, the average soil temperature for winter was coolest in 1999 and 2005 (10.5 and 9.5 °C, respectively) (Figure 1). Throughout the experiment, the average soil temperatures were 11.9 °C in winter, 20.3 °C in spring, 25.4 °C in summer and 15.1 °C in autumn (Fig. 1 and 2).

The soil temperature fluctuated less than the air temperature for each season. The average air temperatures in spring and summer (20.7 and 26.6 °C, respectively) were slightly warmer than the average soil temperatures and cooler in autumn and winter (13.9 and 11.2 °C, respectively).

N requirements

Total N at the end of the experiment was greater than at the beginning at all depths (Table 1). The average yields were larger for wheat followed by sunflower, faba bean and chickpea, respectively (Table 2). The N requirement relative to the corresponding yields was greatest in wheat followed by faba bean, sunflower and chickpea, respectively.

Effect of precipitation and temperature on soil NH₄⁺-N content.

Soil NH₄⁺-N levels and annual precipitation showed a strong positive relationship and were affected by the tillage system (Figure 3). The NT NH₄⁺-N levels increased with precipitation more than for CT. Crop rotations showed the same relationship. The wheat-chickpea NH₄⁺-N levels increased most with rain followed by wheat-wheat, wheat-faba, wheat-sunflower and wheat-fallow (data not shown).

No relationship was observed between NH₄⁺-N content and soil or air temperature.

Effect of the treatments on soil NH₄⁺-N content.

The accumulated NH₄⁺-N for the entire soil profile (0-90 cm) was significant for each treatment except for tillage x rotation (Table 3). The ammonium levels showed no clear pattern throughout the experiment (Figure 4); strong fluctuations in ammonium levels

were recorded. The highest $\text{NH}_4^+\text{-N}$ content was observed in 1997 (144 kg ha^{-1}), and the lowest content was observed in 2005 (27 kg ha^{-1}) (Figure 4).

Throughout the study period, the soil $\text{NH}_4^+\text{-N}$ content was greater in NT than CT (57 and 48 kg ha^{-1} , respectively); significant differences were observed in only five of the thirteen years: 1995, 1997, 2002, 2004 and 2006 (Figure 4). Though crop rotation and the N fertiliser rate were significant (Table 3), the effects were disparate for different years without a particular pattern (data not shown).

Effect of depth on soil $\text{NH}_4^+\text{-N}$ content.

Throughout the study period, the $\text{NH}_4^+\text{-N}$ levels were larger for the deepest soil (30-60 cm and 60-90 cm) compared with the shallowest soil (0-30 cm); the $\text{NH}_4^+\text{-N}$ content was 21.0 , 21.4 and 19.5 kg ha^{-1} , respectively. This trend was observed for the majority of the years studied. However, in 1995, the $\text{NH}_4^+\text{-N}$ levels decreased as the soil profile depth increased, and in 2000, the differences among depths were not significant (Figure 5).

Among the depths, the ammonium level patterns varied between the first (1995) and last years (2009) of the study (Figure 6). In the first year, soil $\text{NH}_4^+\text{-N}$ content decreased progressively with depth (17 , 13 and 10 kg ha^{-1} at 0-30, 30-60 and 60-90 cm, respectively). However, for the last year the soil $\text{NH}_4^+\text{-N}$ content was greater in the deepest layers (21 kg ha^{-1}) compared with the shallowest layer (11 kg ha^{-1}) (Figure 6). Significant differences were not observed for the tillage systems at all depths in the first year (1995) and at 30-60 cm in the last year (2009), when the NT $\text{NH}_4^+\text{-N}$ levels were greater than for CT (Figure 6).

Discussion

The soil $\text{NH}_4^+\text{-N}$ content was strongly related to annual precipitation. Large water contents in the soil facilitated mineralisation. Nitrogen mineralisation increased as the percentage of soil pores filled with water increased to approximately 60% (Power, 1990). However, according to Mikkelsen et al. (1995), mineralisation ends upon $\text{NH}_4^+\text{-N}$ formation under excessive rain and anaerobic conditions in the soil; it is assumed that the oxidative conditions necessary for nitrification are not present. Under such conditions, more $\text{NH}_4^+\text{-N}$ is accumulated than $\text{NO}_3^-\text{-N}$ in the soil.

Total N at the end of the experiment was larger due to the supply of different N rates in wheat plots as mentioned in materials and methods. The N needs of the crops were met depending on treatment.

The largest $\text{NH}_4^+\text{-N}$ soil content was observed in 1997, which together with the previous year (953 mm) had the greatest precipitation (935 mm). Conversely, the smallest $\text{NH}_4^+\text{-N}$ content was recorded in 2005, which had the least precipitation (263 mm). According to Jarvis et al. (1996), soil water content controls solute diffusion and mass distribution of microbial activity products. Low water potentials in the soil may limit biological activity, which in turn limits mineralisation and $\text{NH}_4^+\text{-N}$ levels (Jarvis et al., 1996).

Soil $\text{NH}_4^+\text{-N}$ content were significantly different over the years. According to Jarvis et al. (1996), mineralisation can be highly influenced by precipitation and temperature changes that are typical for field conditions. However, neither soil nor air temperature influenced ammonium levels in this study. According to Li et al. (2013), warmer temperatures support $\text{NH}_4^+\text{-N}$ uptake when the pH is within a medium range (4.0 to 6.5), whereas when the pH ranges from 6.5 to 8.5, the $\text{NH}_4^+\text{-N}$ uptake is unrelated to temperature. The soil in this study was at pH 7.6 (Table 1). According to Li et al. (2013),

soil temperature may not be a strong influence on $\text{NH}_4^+\text{-N}$ uptake by the plant, and consequently, one would not expect accumulated soil $\text{NH}_4^+\text{-N}$ to vary with temperature.

Typically, we would expect less N mineralisation, defined here as transformation of organic N into inorganic forms ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$), for conservation tillage compared with conventional tillage due to reduced soil disturbance, which would yield slower decomposition and N release rates for organic matter in soil (Schoenau & Campbell, 1996). Thus, in a study performed under the conditions of our study, López-Bellido et al. (2013) observed greater $\text{NO}_3^-\text{-N}$ content under CT compared with NT. The soil $\text{NH}_4^+\text{-N}$ content herein were likely greater for NT because such cultivation requires less soil ammonium, as noted in Dong et al. (2012). According to these authors, the microbial biomass and organic material physical protection are less in macroaggregates [in no-tillage] compared with conventional tillage. Typically, soil aeration is greater for CT than NT, especially for Vertisols. Li et al. (2013) notes that $\text{NH}_4^+\text{-N}$ rapidly transforms into $\text{NO}_3^-\text{-N}$ in well-aerated soil, which generates a lower soil $\text{NH}_4^+\text{-N}$ concentration for CT.

Although crop rotation and the nitrogen fertiliser rate were significant compared with soil $\text{NH}_4^+\text{-N}$ content, the different treatments did not produce a clear pattern. For example, it has been suggested that adding immediately available N to fertilisers stimulates mineralisation through a 'priming effect' (Jarvis et al., 1996). However, modelling (Jenkinson et al., 1985) and experimental studies (Hart et al., 1986) have demonstrated that such observations are typically an artefact of the experiment and are not likely significant under most circumstances.

In this study, the soil levels of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ (López-Bellido et al., 2013) or soil organic matter were unrelated for the full profile and at different depths (data not shown).

Ammonium content in the soil was greatest for the deepest layers. According to Page et al. (2002), large ammonium concentrations in subsoil indicates that nitrifying organisms are likely absent or inhibited by soil environmental conditions. It should be noted that certain authors have also speculated that periodic ammonium fixation in clay interlayers (e.g., Vertisols) may reduce nitrification rates by protecting ammonium from nitrifying organisms (Corbeels et al., 1999). Such authors also indicate that Vertisols can produce hydromorphic conditions, especially at deeper levels; little or no oxygen can inhibit nitrifying activity. Another factor that limits nitrification is soil salinity (Murase et al., 1994; Inubushi et al., 1999; Rysgaard et al., 1999) because microbial populations are adversely affected by large salt concentrations (Page et al., 2002). Specifically, nitrifiers are especially sensitive to the osmotic stress on the microbial cell under saline conditions (Inubushi et al., 1999). Herein, the electrical conductivity measured in the soil for 2010 increased with depth and ranged from 0.44 dS m⁻¹ at the surface to 2.4 dS m⁻¹ at 60-90 cm depth due to salinisation induced by the greatest fertiliser rates (López-Bellido et al., 2013). According to Inubushi et al. (1999) and Rysgaard et al. (1999), saline conditions in subsoil may inhibit nitrification.

In addition to the aforementioned causes, ammonium level variations at different depths observed throughout the experiment may be related to the role that cracks play in Vertisols. For the different years as well as alternating dry and wet periods, which are characteristic for the Mediterranean climate, cracks centimetres wide were formed at the soil surface and may extend to depths between 60 and 100 cm (Blokhus, 2006). Organic matter can be displaced through such cracks from the shallowest to the deepest soil layers, which may be related to higher NH₄⁺-N levels.

Conclusions

Soil ammonium content was directly related to annual precipitation; more ammonium was observed in the rainiest years, and less ammonium was observed in the dry years.

The NT produced greater soil $\text{NH}_4^+\text{-N}$ levels than CT possibly because NT can result in lower microbial biomass and organic material physical protection within macroaggregates compared with CT. In addition, Vertisols with CT are typically more aerated than for NT, which results in more rapid transformation from $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ and reduces $\text{NH}_4^+\text{-N}$ soil content.

The deepest soil (30-60 and 60-90 cm) had more $\text{NH}_4^+\text{-N}$ than shallow soil (0-30 cm) likely due to more limited or inhibited nitrification in the deepest layers. Anaerobiosis can occur in the deepest layers due to hydromorphic conditions, which are typical for Vertisols. High soil salinity in the deepest layers can negatively affect microbial populations. In conclusion, cracks that typically form in Vertisols contribute to organic matter displacement from the soil surface to the deepest layers.

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Table 1. The properties of the Vertisol used in field experiments. Córdoba (Spain).

	Soil depth (cm)		
	0-30	30-60	60-90
Fine sand (g kg ⁻¹)	127	143	187
Silt (g kg ⁻¹)	179	152	26
Clay (g kg ⁻¹)	694	705	787
pH (water)	7.7	7.6	7.6
Organic matter (g kg ⁻¹)	10.2	7.4	5.3
Calcium carbonate equivalent (g kg ⁻¹)	75	93	71
CEC (cmol kg ⁻¹)	46.5	36.6	30
Available P (mg kg ⁻¹)			
Beginning of the experiment	4.9	1.5	1.5
End of the experiment	9.1	7.2	5.2
Total N (%)			
Beginning of the experiment	0.07	0.06	0.04
End of the experiment	0.1	0.07	0.06

Table 2. Grain yield and N requirement of crops over 13-year period.

Crop	Yield (kg ha ⁻¹)	N requirement (kg ha ⁻¹)
Wheat	3096	120
Sunflower	1833	77
Faba bean	1640	85
Chickpea	1302	69

Table 3. Analysis of variance (mean squares) of the soil ammonium content at sowing as affected by year, tillage system, crop rotation and N rate over 13-year period.

Source	df	Mean squares
Year (Y)	12	115408***
Tillage (T)	1	32810***
Y × T	12	6203***
Rotation (R)	4	1383*
Y × R	48	762**
T × R	4	506
Y × T × R	48	705*
N rate (N)	3	660*
Y × N	36	572***
T × N	3	694*
R × N	12	440*
Y × T × N	36	413**
Y × R × N	144	350***
T × R × N	12	451*
Y × T × R × N	144	439***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level

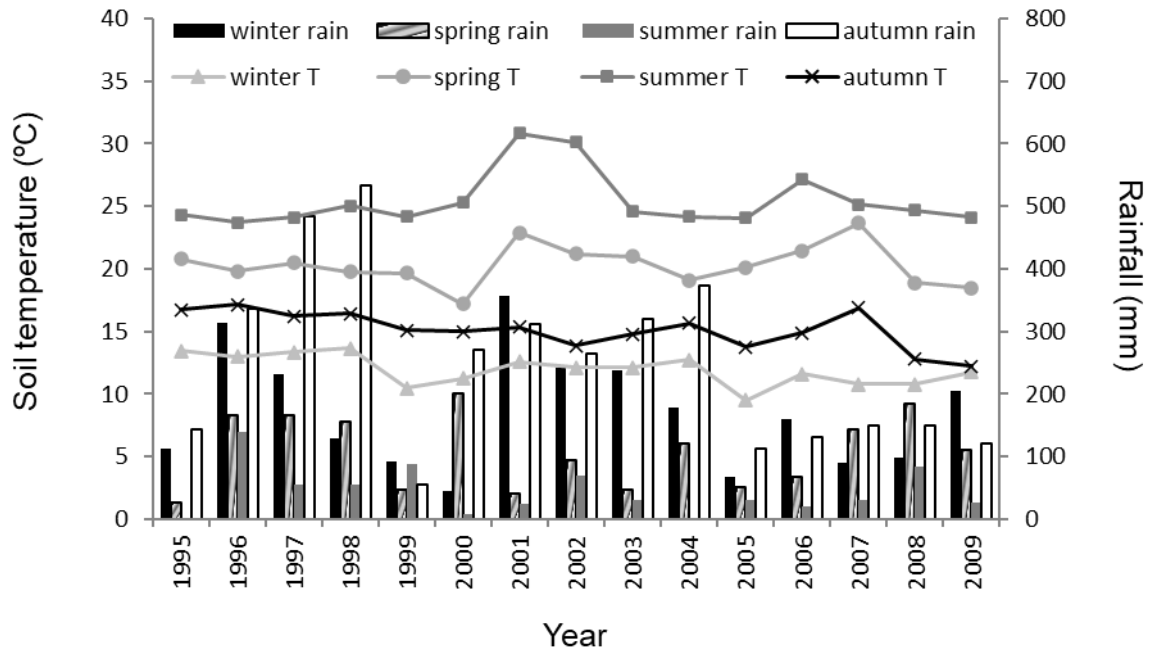


Fig. 1. Season rainfall and soil temperature over the 13-year study at Córdoba (Spain).

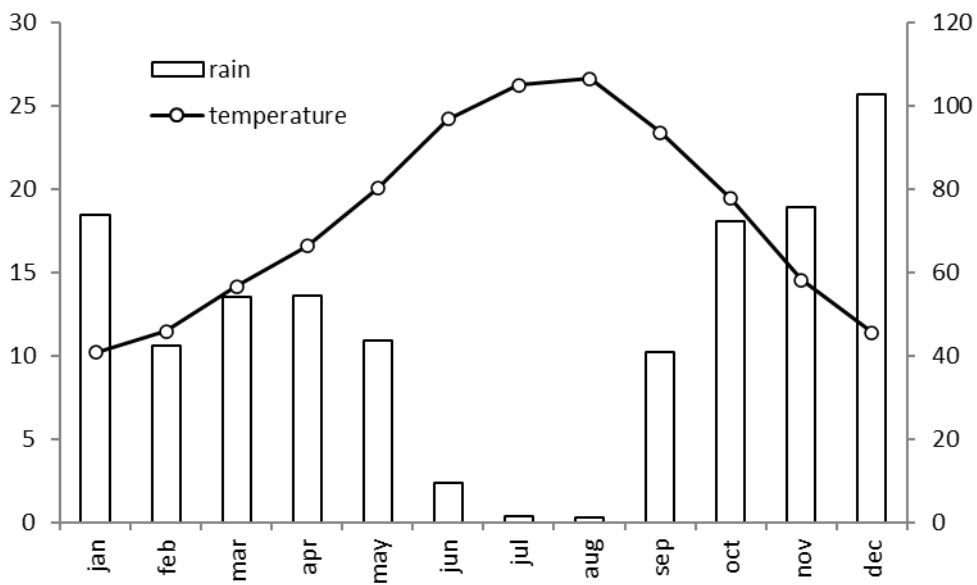


Fig. 2. Monthly rainfall and soil temperature averaged over the 13-year study at Córdoba (Spain).

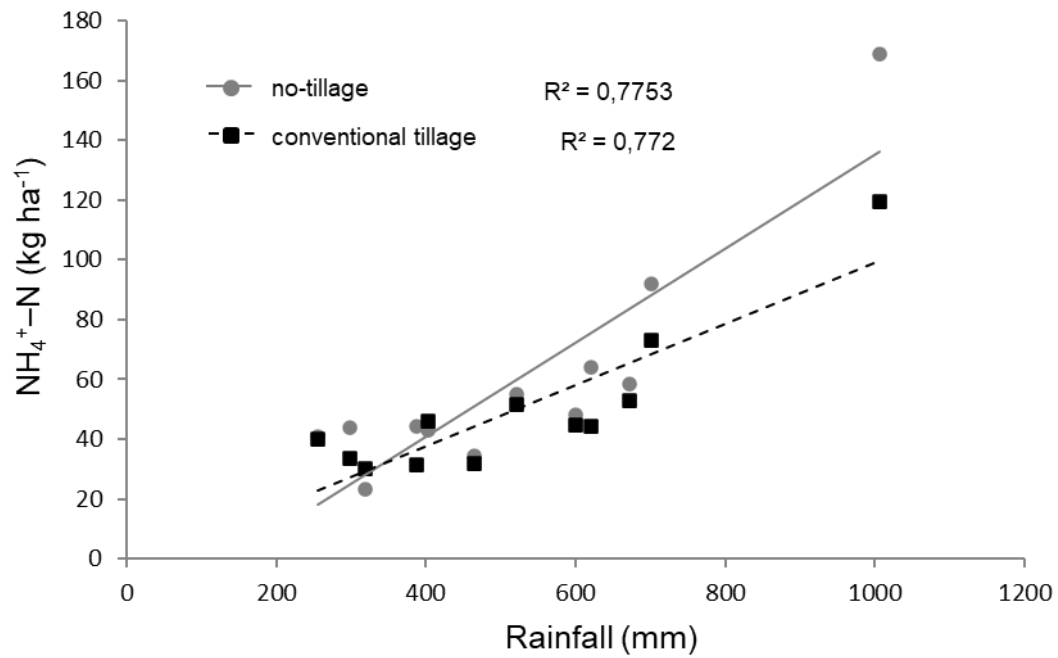


Fig. 3. Relationship between annual rainfall and soil ammonium content in the soil profile (0-90 cm) as affected by tillage system at Córdoba (Spain).

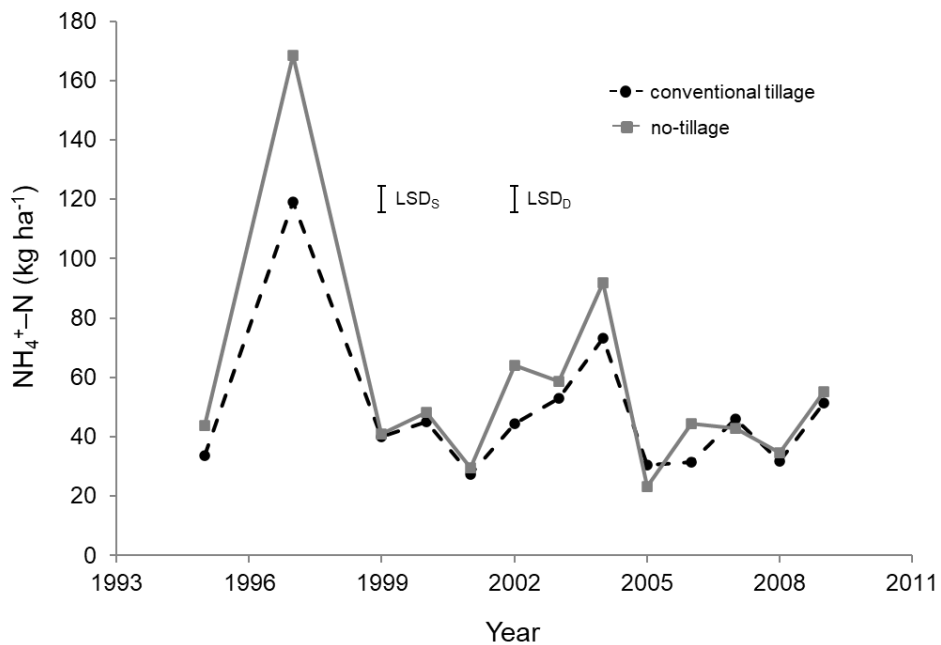


Fig. 4. Development of soil nitrate content in the soil profile (0-90 cm) as affected by tillage system for a 13-year experiment at Córdoba in Spain. Vertical bars represent LSD for comparison: LSD_s, same level of year; LSD_D, different level of year.

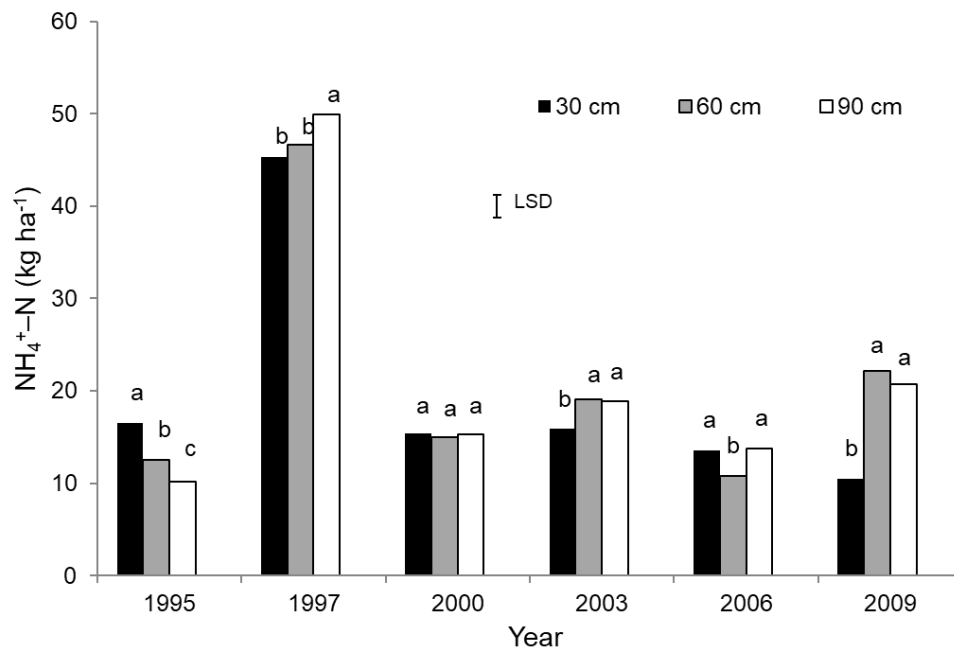


Fig. 5. Soil ammonium content as affected by year and soil depth. For each year means followed by the same letter are not significantly different at $P < 0.05$ according to LSD. Vertical bar represents LSD for different level of year.

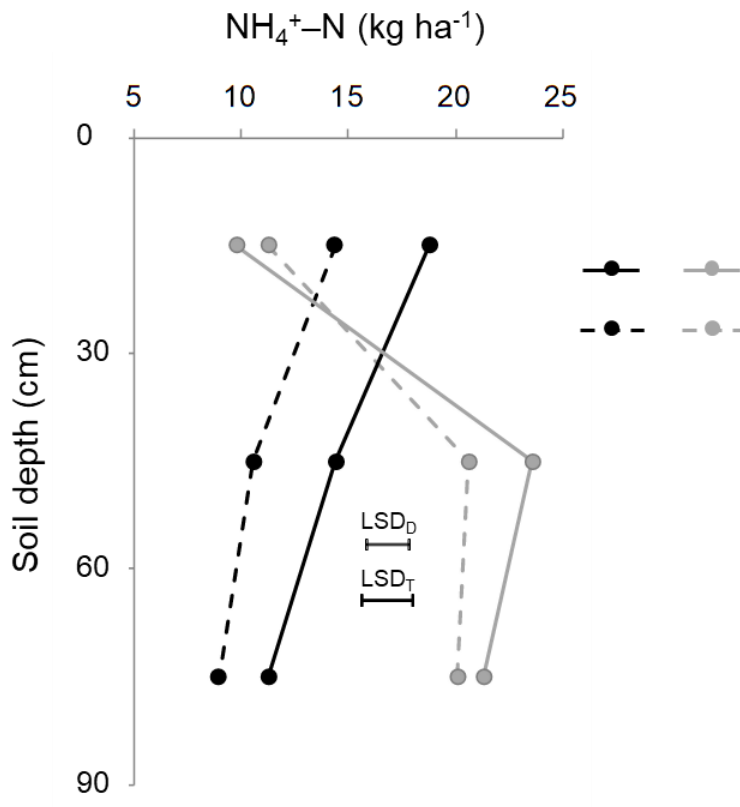


Fig. 6. Soil ammonium content as affected by tillage system (NT, no-tillage; CT, conventional tillage) and soil depth for the first and the last year of the experiment. Horizontal bars represent LSD for comparison: LSD_D , for the same year and tillage; LSD_T , for the same year.