

# In situ net N mineralisation and nitrification under organic and conventionally managed olive oil orchards

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**Abstract** Olive oil orchard occupies a great percentage of the cropland in southern Spain. Thus, changes in nitrogen (N) fertilization might have a great effect on N dynamics at least at regional scale, which should be investigated for a sustainable N fertilization program. In situ net N mineralization (NM) and nitrification (NN) were investigated during a year in comparable organic (OR) and conventional (CV) olive oil orchards of two locations differing their N input. Soil samples were collected in two soil positions (under and between trees canopy) and both buried-bags and soil core techniques were used to quantify both microbial rates. There were differences in NM and NN between sites mainly due to differences in soil total N (TN), and potential mineralisable N (PMN). In all cases NM and NN were higher in soils under tree canopy. NM and NN

were higher in OR than in CV managed orchards in the location with high soil TN. Soil TN and PMN explained together a 50 % of the variability in soil N availability, which suggests that these two variables are good predictors of the potential of a soil to provide available N. The highest rates of soil N availability were found in spring, when olive tree demand for N was at its maximum. Annual soil N availability in olive groves was in all cases higher or similar than tree demand suggesting that soil annual supply of N should be taken into account in order to develop sustainable N fertilisation strategies for olive crops.

**Keywords** Organic and conventional olive crop · Soil N mineralization · N dynamic · Fertilisation

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## Introduction

The maintenance of the amount and availability of soil nutrients for plants, particularly nitrogen (N), is a key component of crop management. Soil nutrient status depends on numerous factors, e.g. soil quality, environmental conditions such as temperature and rainfall, and management practices such as tillage, irrigation and fertilisation (Campbell and Biederbeck 1972; McGill and Myers 1987; Tilman et al. 2002).

An optimal N fertilisation program should be based on achieving synchrony between plant demand for N and soil N availability, not only in magnitude but also

in timing (Tilman et al. 2002). This is a difficult task since the soil N cycling is characterised by many natural soil N transformations and both N input and output processes, which are sensitive to management practices. An accurate knowledge of the rates of these processes and the effects of management practices is essential to enable an optimum N fertilisation.

N mineralisation is a major process supplying mineral N to plants in terrestrial ecosystems (Vitousek and Howarth 1991). The seasonal pattern of soil N mineralisation may regulate soil fertility (Xu et al. 2007), affecting plant growth (Nadelhoffer et al. 1985; Antil et al. 2001) and being responsible for variations in primary production (Burke et al. 1997). Thus, net N mineralisation (NM), net nitrification (NN) and other microbiologically mediated soil processes involved in releasing inorganic N to soil should be taken into account in the fertiliser management of agroecosystems. The magnitude and timing of these processes in agroecosystems are highly dependent on soil type, climate conditions and organic N content of soils.

In agroecosystems, the N cycle is strongly affected by the use of mineral and organic fertilisers, which have a central role in sustaining crop productions (Jarvis et al. 1996; Tilman et al. 2002). In conventional crops, the addition of nutrient is carried out mainly by inorganic fertilisers, which are available in the short-term for plant uptake but they are also liable to be lost by leaching and denitrification. On the other hand, the nutrients uptaken by plants in organic crops comes from organic fertilisers such as compost, plant residues or green manures, which increase the organic matter content in soil, but they must be previously mineralised to be available for the plants. However, soil with high organic matter inputs have greater labile carbon (C) pools, greater microbial activity and greater ability to provide inorganic N through mineralization compared to agroecosystems that receive only inorganic fertilisers (Gunapala and Scow 1998; Kramer et al. 2002).

Globally the olive crops occupy around 10 million of hectares, 98 % of which are located in the Mediterranean region (Civantos 2008). Over the last 10 years, the surface of organic olive crops has increased by about 25 % and is expected to continue increasing due to the EU agricultural policy implementation. Generally, in conventional olive crops the addition of mineral fertilisers, mainly N, tends to be above optimal levels in order to avoid their limitation

for crop growth. In fact, Fernández-Escobar (2011) showed that over-fertilisation is a general feature of the olive crops in Spain. This excess of soil N is associated with a decline of nutrient-use efficiency of crops and several environmental impacts (Raun and Schepers 2008). Thus, in order to reduce the over-fertilisation and the environmental damages associated with these practices it is necessary to predict the N needed by crops (Gutser et al. 2005). Different studies have established fertilisers recommendations based on nutrients removed by yield, pruning or leaf analysis (Rodrigues et al. 2012; Righetti et al. 1990; Fernández-Escobar et al. 2009). However, few studies have considered N mineralisation of native soil as an inputs of available N for the olive orchards in order to establish an efficient protocol of N fertilisation.

Usually the amount of inorganic N supply by native soil throughout N mineralisation has been estimated by means of aerobic incubations under controlled conditions of the laboratory (Stanford and Smith 1972). However, this method has some limitations when transferring results at field conditions, since incubations conditions make the rate of this process optimal. In addition, soil is disturbed through storing, mixing and sieving (Curtin and McCallum 2004).

On the other hand, several techniques have been proposed to estimate the NM under field conditions, among which the following are the most popular: (1) the buried-bag (Eno 1960); (2) the covered-cylinder (Hatch et al. 1998; Durán et al. 2012) and (3) the resin-trap techniques (Di Stefano and Gholz 1986). All in situ incubation techniques suffers also from problems such as disturbance of the soil prior to the incubation, increase of oxygen content, physical isolation, differences in environmental conditions inside and outside or the choice of the duration of incubation (Stenger et al. 1996; Hanselman et al. 2004). Furthermore, the great variability of conditions found in the field involves that a high number of replicates is required. Although the buried-bags technique is considered less accurate than others in situ techniques, it is the simplest one, only causes moderate disturbance of the soil, and allows investigation of N dynamics of subsurface soil layers; which makes this method suitable for agronomic investigations (Monaco et al. 2010).

The main objective of this work was to evaluate the magnitude and timing of soil inorganic N supply by soil MN and NN of olive orchards under different

management practices (organic versus conventionally managed) and at different soil location within the orchard (under and between tree canopy). In order to carry out our objective both buried-bag and soil core techniques were used to quantify both microbial rates.

## Materials and methods

### Field sites

This study was conducted in the southwest of Andalucía (Spain). Two organic olive orchards (OR) differing in soil and landscape features were selected from the databases supplied by CAAE (Certification Institution for Organic Production).

One of them, an olive oil farm subjected to organic management during the last 10 years, it was located in Deifontes (D site, hereafter), Granada (37°16′06″; 3°34′14″). The olive trees were 19 years old with a plantation density of about 165 trees ha<sup>-1</sup>. The soil was Calcium Cambisol (IUSS Working Group WRB, 2007) in a predominant low slope landscape. The main management practices were (1) low tillage intensity (one or two times per year); (2) natural plant growing in the intercanopy gaps and (3) nutrient replacement was based on sheep manure application. The intercanopy area was covered by plants from May to the following March, when they were mowed. In this orchard two subplots (1,500 m<sup>2</sup>) were selected with two types of plant cover, i.e. with both natural plants and seeded with *Vicia* sp. (150 kg of seeds ha<sup>-1</sup>). However, soil inorganic N and in situ NM and NN rates were not significantly different between subplots with natural plants and seeded *Vicia* sp. covers (one way ANOVA,  $p > 0.54$ ) along the studied period (data not shown). Thus, data collected from the subplot covered by *Vicia* sp. were considered as replicates of the subplot covered by natural plants. Sheep manure is biannually applied under tree canopy (UTC) nearby the tree trunk in late winter, at a rate of 6–8 tonnes (wet weight averaged 23 %) containing on average 0.53 % of total N (wet weight). Thus, about 31.8–42.4 kg N ha<sup>-1</sup> has been applied biannually. In addition, drip irrigation was applied in summer UTC.

The other organic olive oil orchard, Cortijo Tobazo (CTO site, hereafter), was selected in Alcaudete (Jaén) (37°33′05″; 4°01′33″). Olive trees were about 40 years old with a low-to-medium plantation density of about

68–70 trees ha<sup>-1</sup>. The soil was classified as Vertisol (IUSS Working Group WRB 2007) in a medium slope landscape. The main organic management practices set up during the last 10 years were: (1) no tillage, (2) natural plant cover was allowed to grow from May to March, when it was controlled by mowing and, (3) nutrient replacement was based on composted olive mill pomace application. Composted olive mill pomace was applied annually in the intercanopy gaps at a rate of about 4 tonnes ha<sup>-1</sup> (wet weight, average water content of 19.3 %) on October–November period. On average for the last 3 years, composted olive mill pomace contained 0.82 % N. Then, this farm received annually about 32.8 kg N ha<sup>-1</sup>.

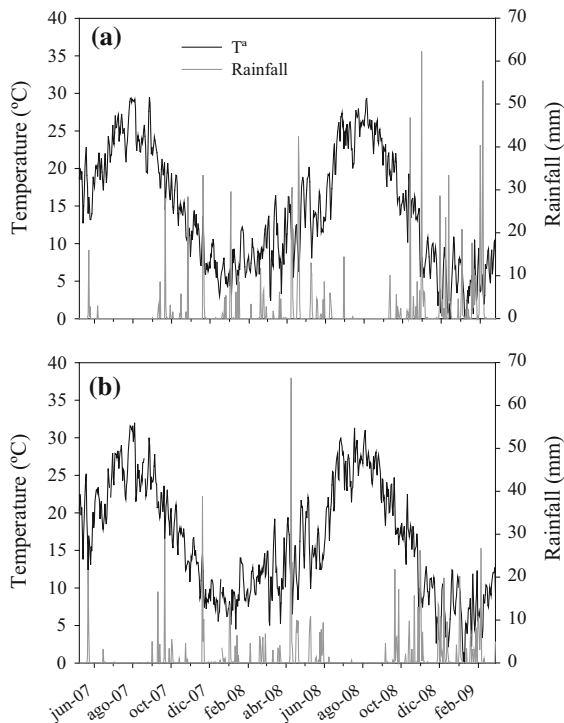
Conventionally managed olive oil farms (CV sites, hereafter) with similar environmental conditions, landscape feature, tree age and density and soil types (Cambisol in D and Vertisol in CTO) and properties were selected nearby (<20 m) of each organic olive farms. Their management practices consisted of: (1) chemical fertilisation (ammonium nitrate or urea) UTC and (2) bared soil in the intercanopy area of the farms by intensive tillage (2–3 times per year) in combination with pre and post emergency herbicides.

Thus, experimental plots consisted of two study sites (D and CTO) and two comparable olive oil farms of contrasting management (OR and CV) at each site and two locations within each farm (under canopy and intercanopy soils).

Both sites (D and CTO) have a continental Mediterranean climate. The average temperature in the sampling period was 16.1 and 18.1 °C for D and CTO sites, respectively. During the studied period the accumulated annual rainfall was 625.8 and 555.4 mm in D and CTO sites, respectively. Temporal patterns of temperature and rainfall were similar for the two sites, except for the period from October 2008–February 2009, in which D site recorded a higher rainfall (Fig. 1).

### Soil characterization

Soil samples of the two organic (OR) and the two comparable conventional olive orchards (CV) in D and CTO sites were collected in two different tree canopy positions; under-(UTC) and between (BT) tree canopy for an initial soil characterization. In all plots four replicate soil samples were taken from the surface (0–10 cm) and subsurface (10–20 cm). Samples were transported to the laboratory on the same day and



**Fig. 1** Daily mean temperature (°C) and rainfall (mm) during the field experiment in the study sites: **a** Deifontes and **b** Cortijo Tobazo

stored at 4 °C until their analysis. Soils samples were analysed for water content estimated by gravimetry (Gardner 1986). Soil bulk density was analysed according to Blake and Hartge (1986). Particle size distribution was determined by the pipette method (Gee and Bauder 1986). Cation exchange capacity (CEC) was analyzed according to Rhoades (1982). Air-dried subsamples were used to analyze exchangeable bases content (Grant 1982), and soil available phosphorus (Olsen and Sommers 1982). Soil organic matter (OM) was estimated according to Nelson and Sommers (1982). An aliquot of the dried soil was ground to a fine powder (<1 mm), and soil total nitrogen (TN) and total carbon (TC) was analyzed using a Leco CNH-932 analyzer. Soil organic carbon (SOC) was determined from organic matter using a conversion factor of 1.724, based on the assumption that organic matter contains approximately 58 % organic C (Nelson and Sommers 1982). The pool of easily mineralised nitrogen (PMN) in soil was analyzed according to Kandeler (1995). Table 1 shows the main properties of soils present under each experimental treatment.

Furthermore, in order to assess the nutrient content according to particle-size ranges, the percentage of soil particles >4 mm, between 2 and 4 mm and lower than 2 mm was determined in the soil collected in D site by sieving successively through different pore size mesh. Soil of these fractions was analysed for % moisture, TN, TC and PMN using the methods described above. Net N mineralisation (NM) and net N nitrification (NN) were determined in a short-term aerobic incubation in the laboratory (1 month) at optimal condition of temperature (25 °C) and at 60 % WHC. Soil samples were extracted with 2 M KCl before and after incubation to determine the inorganic N (Keeney and Nelson 1982), and the NM and NN was calculated using the equation proposed by Hart et al. (1994).

#### In situ soil net N mineralisation (NM) and net N nitrification (NN) rate measurements

The temporal pattern of soil NM and NN rates were measured in situ in each experimental plot using the buried-bag technique as described by Hart et al. (1994). Plastic bags were used for this purpose as they are permeable to gases allowing for gas exchange, but impermeable to liquids (Gordon et al. 1987). This technique integrates on-site soil water dynamics but only if the soil water content at the beginning of the incubation period is representative of soil water conditions for the entire incubation period. Eight top soil (10 cm) subsamples were sampled (using a core, 5 cm diameter and 12 cm height) from each site and sieved (2 mm) in the field. One set of four soil subsamples (about 40 g of fresh soil), considered as initial soil for the studied period, was transported to the laboratory. The other set of four subsamples was placed in polyethylene bags, tied shut, and buried in the hole from which soil was taken and incubated. After a month, the incubated samples were transported to the laboratory and a new soil samples were taken, repeating the procedure 13 times from September 2007 to October 2008.

In addition, the intact soil core incubation technique was used, together with the buried-bag technique, at least once per season, in order to test any correlation between the rates of NM and NN estimated using both techniques. At each study plot and position intact soil cores (5 × 12 cm) were taken and transported to the

**Table 1** Main soil properties of organic (OR) and conventional (CV) orchards sampled between tree (BT) and under tree (UTC) canopy in Deifontes (D) and Cortijo Tobazo (CTO). Organic matter (OM), total N (TN), soil organic C (SOC), organic C:N ratio and potential mineralizable N (PMN) are shown for two depths (0–10 and 10–20 cm) in the same sites

	Deifontes				Cortijo Tobazo			
	Organic		Conventional		Organic		Conventional	
	UTC	BT	UTC	BT	UTC	BT	UTC	BT
Texture	Loam	Loam	Loam	Loam	Clay-Loam	Clay	Clay-Loam	Clay
Clay (%)	16.6 ± 0.46 <sup>a</sup>	18.2 ± 0.32 <sup>b</sup>	24.9 ± 1.19 <sup>c</sup>	24.1 ± 0.73 <sup>c</sup>	39.0 ± 0.21 <sup>d</sup>	41.6 ± 0.65 <sup>e</sup>	34.0 ± 2.34 <sup>f</sup>	55.4 ± 1.52 <sup>g</sup>
Sand (%)	48.9 ± 1.29 <sup>a</sup>	45.3 ± 1.29 <sup>b</sup>	44.2 ± 3.93 <sup>b</sup>	41.4 ± 0.97 <sup>c</sup>	30.7 ± 1.54 <sup>d</sup>	27.6 ± 1.07 <sup>e</sup>	34.7 ± 2.41 <sup>f</sup>	19.9 ± 1.85 <sup>g</sup>
Silt (%)	34.5 ± 1.20 <sup>a</sup>	36.4 ± 1.61 <sup>b</sup>	30.9 ± 3.12 <sup>c</sup>	34.6 ± 1.20 <sup>a</sup>	30.3 ± 1.42 <sup>c</sup>	30.7 ± 0.46 <sup>c</sup>	31.3 ± 2.03 <sup>c</sup>	24.7 ± 2.79 <sup>d</sup>
Bulk density(g cm <sup>-3</sup> )	0.99 ± 0.27 <sup>a</sup>	1.66 ± 0.11 <sup>b</sup>	1.37 ± 0.14 <sup>ab</sup>	1.88 ± 0.72 <sup>b</sup>	1.45 ± 0.35 <sup>ab</sup>	1.78 ± 0.47 <sup>b</sup>	1.69 ± 0.35 <sup>b</sup>	2.66 ± 0.51 <sup>c</sup>
CEC (meq/100 g)	25.1 ± 0.25 <sup>a</sup>	19.9 ± 0.45 <sup>b</sup>	21.8 ± 1.37 <sup>c</sup>	19.1 ± 0.85 <sup>b</sup>	20.1 ± 1.20 <sup>b</sup>	19.3 ± 0.54 <sup>b</sup>	17.2 ± 1.37 <sup>d</sup>	35.5 ± 0.65 <sup>e</sup>
Exchangeable Ca (meq/100 g)	12.7 ± 0.62 <sup>ab</sup>	14.0 ± 1.59 <sup>b</sup>	16.0 ± 0.84 <sup>c</sup>	13.1 ± 0.37 <sup>ab</sup>	13.5 ± 2.52 <sup>b</sup>	11.6 ± 0.79 <sup>d</sup>	11.0 ± 1.86 <sup>d</sup>	27.1 ± 1.13 <sup>e</sup>
Exchangeable Mg (meq/100 g)	7.22 ± 1.31 <sup>a</sup>	4.51 ± 1.57 <sup>bc</sup>	3.97 ± 1.80 <sup>c</sup>	4.48 ± 1.17 <sup>bc</sup>	4.42 ± 1.73 <sup>bc</sup>	5.85 ± 0.81 <sup>ab</sup>	4.40 ± 1.46 <sup>bc</sup>	6.44 ± 1.59 <sup>a</sup>
Exchangeable Na (meq/100 g)	1.02 ± 0.44 <sup>a</sup>	0.36 ± 0.02 <sup>b</sup>	0.34 ± 0.01 <sup>b</sup>	0.37 ± 0.03 <sup>b</sup>	0.33 ± 0.02 <sup>b</sup>	0.36 ± 0.01 <sup>b</sup>	0.36 ± 0.04 <sup>b</sup>	0.35 ± 0.02 <sup>b</sup>
Exchangeable K (meq/100 g)	4.08 ± 0.31 <sup>a</sup>	1.10 ± 0.14 <sup>b</sup>	1.46 ± 0.28 <sup>cde</sup>	1.16 ± 0.26 <sup>c</sup>	1.86 ± 0.03 <sup>c</sup>	1.46 ± 0.06 <sup>cde</sup>	1.44 ± 0.76 <sup>cde</sup>	1.68 ± 0.60 <sup>cd</sup>
Carbonate (%)	41.4 ± 0.39 <sup>a</sup>	41.2 ± 1.45 <sup>a</sup>	30.4 ± 0.84 <sup>b</sup>	42.3 ± 3.28 <sup>ac</sup>	29.4 ± 0.84 <sup>b</sup>	30.5 ± 1.64 <sup>b</sup>	43.5 ± 3.50 <sup>c</sup>	5.16 ± 1.24 <sup>d</sup>
P Olsen (ppm)	65.7 ± 4.94 <sup>a</sup>	22.0 ± 2.25 <sup>bcd</sup>	34.5 ± 3.91 <sup>cde</sup>	43.6 ± 9.63 <sup>c</sup>	14.3 ± 1.84 <sup>b</sup>	7.63 ± 0.99 <sup>b</sup>	21.4 ± 24.4 <sup>bcd</sup>	36.7 ± 33.6 <sup>de</sup>
OM (%)	5.40 ± 0.74 <sup>a</sup>	6.08 ± 1.76 <sup>a</sup>	5.55 ± 0.47 <sup>a</sup>	6.16 ± 1.53 <sup>ab</sup>	6.22 ± 1.42 <sup>ab</sup>	5.59 ± 2.36 <sup>a</sup>	7.25 ± 0.88 <sup>b</sup>	5.59 ± 1.68 <sup>a</sup>
	4.02 ± 0.17 <sup>ab*</sup>	5.73 ± 0.14 <sup>c*</sup>	3.28 ± 0.49 <sup>b*</sup>	3.98 ± 0.13 <sup>ab*</sup>	6.54 ± 0.67 <sup>d</sup>	4.84 ± 1.88 <sup>ac</sup>	4.58 ± 1.09 <sup>a*</sup>	7.03 ± 0.65 <sup>d*</sup>
TN (%)	0.40 ± 0.09 <sup>a</sup>	0.20 ± 0.07 <sup>bc</sup>	0.19 ± 0.04 <sup>bc</sup>	0.18 ± 0.04 <sup>bcd</sup>	0.19 ± 0.06 <sup>bc</sup>	0.16 ± 0.07 <sup>cd</sup>	0.20 ± 0.06 <sup>bc</sup>	0.13 ± 0.06 <sup>d</sup>
	0.25 ± 0.09 <sup>a*</sup>	0.15 ± 0.04 <sup>bc*</sup>	0.16 ± 0.05 <sup>bc</sup>	0.15 ± 0.04 <sup>bcd</sup>	0.18 ± 0.06 <sup>c</sup>	0.16 ± 0.06 <sup>bc</sup>	0.13 ± 0.03 <sup>bd*</sup>	0.10 ± 0.04 <sup>d</sup>
SOC (%)	3.1 ± 0.30 <sup>a</sup>	3.5 ± 0.83 <sup>ab</sup>	3.2 ± 0.27 <sup>a</sup>	3.6 ± 0.67 <sup>ab</sup>	3.6 ± 0.82 <sup>ab</sup>	3.2 ± 1.12 <sup>a</sup>	4.2 ± 0.40 <sup>b</sup>	3.2 ± 0.76 <sup>a</sup>
	2.3 ± 0.1 <sup>ab*</sup>	3.3 ± 0.1 <sup>c</sup>	1.9 ± 0.3 <sup>b*</sup>	2.3 ± 0.1 <sup>ab*</sup>	3.8 ± 0.3 <sup>c</sup>	2.8 ± 0.9 <sup>a</sup>	2.7 ± 0.5 <sup>a*</sup>	4.1 ± 0.3 <sup>a*</sup>
Corg:N	8.5 ± 1.61 <sup>a</sup>	16.4 ± 1.29 <sup>bc</sup>	16.8 ± 2.15 <sup>bc</sup>	21.1 ± 9.57 <sup>bc</sup>	19.9 ± 8.83 <sup>bc</sup>	26.1 ± 20.04 <sup>b</sup>	22.1 ± 4.55 <sup>bc</sup>	27.4 ± 10.2 <sup>b</sup>
	9.3 ± 0.9 <sup>a</sup>	24.2 ± 1.7 <sup>b</sup>	12.0 ± 2.5 <sup>ac</sup>	16.0 ± 2.3 <sup>abc</sup>	21.0 ± 6.2 <sup>b</sup>	21.8 ± 14.6 <sup>b</sup>	22.5 ± 6.8 <sup>b</sup>	42.1 ± 6.8 <sup>d*</sup>
PMN (μg N-NH <sub>4</sub> <sup>+</sup> g <sup>-1</sup> )	102 ± 55 <sup>a</sup>	95 ± 36 <sup>a</sup>	56 ± 21 <sup>bc</sup>	57 ± 26 <sup>bc</sup>	71 ± 32 <sup>b</sup>	56 ± 45 <sup>bc</sup>	45 ± 39 <sup>bc</sup>	31 ± 18 <sup>d</sup>
	49.3 ± 36.7 <sup>ab*</sup>	51.3 ± 25.4 <sup>b*</sup>	31.4 ± 17.0 <sup>c*</sup>	28.2 ± 17.8 <sup>ac*</sup>	56.0 ± 36.9 <sup>b</sup>	29.2 ± 24.7 <sup>ac*</sup>	31.4 ± 32.0 <sup>ac</sup>	12.3 ± 10.2 <sup>c</sup>

Data are the mean ± SD (n = 5). Different letters in the same row denote significant differences ( $p < 0.05$ ) among soils of different positions, management and sites (D and CTO)

\* Significant differences ( $p < 0.05$ ) between depths for the same position, management and site

laboratory (initial) and another stainless steel core was placed on soil and incubated at field condition during a month period.

In the laboratory, soil nitrate and ammonium concentration were analysed on soil samples at the beginning and at the end of each of the incubation periods for both buried-bag and intact soil cores methods (Keeney and Nelson 1982). The NM and NN rates were calculated following the equation described below (Hart et al. 1994).

$$\text{NM}(\mu\text{gN g}^{-1}\text{day}^{-1}) = \frac{[\text{NO}_3^- + \text{NH}_4^+]_{\text{final}} - [\text{NO}_3^- + \text{NH}_4^+]_{\text{initial}}}{\text{Incubation time (days)}}$$

$$\text{NN}(\mu\text{gN g}^{-1}\text{day}^{-1}) = \frac{[\text{NO}_3^-]_{\text{final}} - [\text{NO}_3^-]_{\text{initial}}}{\text{Incubation time (days)}}$$

The net amount of N mineralised and nitrified were expressed per hectare (10 cm depth) after corrections taking into account: (1) the percentage of soil particles lower than 2 mm, (2) and the soil bulk density and (3) the percentage of area of BT and UTC position at each site.

TC, TN and PMN were analysed once per season following the methods described above.

### Statistical analysis

Differences among sites (D and CTO), managements (OR and CV) and soil positions (UTC and BT) on general soil physico-chemical and biological variables were tested by factorial ANOVA. The overall effects of sites, managements, and soil position on soil level of inorganic N, net NM rate and inorganic N produced by mineralization at different samplings along the year, were tested by repeated measures ANOVA. Differences among levels of each factor were tested using the Fisher Least Significant Difference (LSD) test. Assumptions for analysis of variance (homogeneity and normality) were tested and assured by using transformed data sets [ $\log(\text{dependent variable} + 1)$ ] when it was necessary. Correlation among the studied variables was tested by the Pearson-moment correlation coefficient. Forward multiple regression analysis was used to predict the N mineralisation from the soil properties. Significance was accepted at  $p < 0.05$  in all cases.

## Results

### Particle size fractions effects on soil N

Overall, between 50.8 and 61.2 % of soil was composed of particles  $<2$  mm, whereas a low proportion ( $<10.6$  %) corresponded to particles between 2 and 4 mm, and between 28.9 and 39.5 % of the soil particles were  $>4$  mm (Table 2). Management and depth factors did not have any significant effect on the relative proportion of the soil particles. TN concentration in the  $<2$  mm fraction was typically between 2 and 3.5 times higher than the other fractions. Similarly, the potential for N mineralisation (PMN) was significantly higher in the smallest fraction ( $<2$  mm) for all cases. However, the decrease of PMN in the larger soil particles was more marked in organic than in the conventional soils. The amount of mineral N was similar in the soil samples from OR and CV managements, and in all cases the content of mineral N was significantly lower in the largest particles. Net N mineralisation (NM) rate was positive for particle size smaller than 2 mm and ranged from 0.36 to  $3.36 \mu\text{g N g}^{-1} \text{day}^{-1}$ . Figures were between three and ten times lower in the 2–4 mm size fraction and were negative or close to zero in the largest size fraction. Independently of the management and depth, the highest content of N ( $>75$  %) was observed in the smaller fraction ( $<2$  mm), whereas the greater soil fractions accounted by between 15 and 20 % of the total N. Similar results were found for the whole soil content of potential mineralisable N (PMN). 98–100 % of the mineralised N was due to microorganism linked to soil particles smaller than 2 mm, and typically it was 5 times higher for 0–10 cm soils of depth compared with 10–20 cm soil (Table 2).

### Soil mineral N dynamic

Overall, soil ammonium concentration was very low ( $\approx 5 \mu\text{g N-NH}_4^+ \text{g}^{-1}$ ) for all periods, managements and soil positions and typically it was lower than 10 % of the soil mineral nitrogen (nitrate plus ammonium) concentration. The overall temporal pattern of soil mineral N was similar for OR and CV orchards in D site, with relatively higher values during spring and summer and the lowest during winter sampling. In CTO site there was an unclear temporal pattern,



**Table 2** Total N (TN) and C (TC), mineral N, potential mineralisable N (PMN) and net N mineralisation (NM) of three particle size fractions of soil at two depths (0–10 cm and 10–20 cm) in both organic and conventional olive oil orchards in Deifontes (D)

Management	Depth (cm)	Fraction (mm)	Relative proportion	TN (%)	TC (%)	Mineral N ( $\mu\text{g g}^{-1}$ )	PMN ( $\mu\text{g N-NH}_4^{+}\text{g}^{-1}$ )	NM ( $\mu\text{g N g}^{-1}\text{day}^{-1}$ )
Organic	0–10	<2	58.5 $\pm$ 6.6 <sup>Aa</sup>	0.27 $\pm$ 0.06 <sup>Aa</sup>	9.24 $\pm$ 0.93 <sup>Aa</sup>	31.7 $\pm$ 15.2 <sup>Aa</sup>	72.1 $\pm$ 29.1 <sup>Aa</sup>	3.36 $\pm$ 0.67 <sup>Aa</sup>
		2–4	9.0 $\pm$ 0.8 <sup>Ba</sup>	0.08 $\pm$ 0.03 <sup>Ba</sup>	10.1 $\pm$ 0.77 <sup>Aa</sup>	39.6 $\pm$ 4.8 <sup>Aa</sup>	23.1 $\pm$ 8.5 <sup>Ba</sup>	0.34 $\pm$ 0.64 <sup>Ba</sup>
		>4	32.4 $\pm$ 6.6 <sup>Ca</sup>	0.02 $\pm$ 0.01 <sup>Ba</sup>	10.3 $\pm$ 1.27 <sup>Aa</sup>	13.1 $\pm$ 9.3 <sup>Ba</sup>	11.6 $\pm$ 4.8 <sup>Ba</sup>	−0.04 $\pm$ 0.08 <sup>Ba</sup>
	10–20	<2	61.2 $\pm$ 3.3 <sup>Aa</sup>	0.17 $\pm$ 0.03 <sup>Aa</sup>	8.27 $\pm$ 1.31 <sup>Aa</sup>	21.4 $\pm$ 10.1 <sup>Aa</sup>	16.3 $\pm$ 5.7 <sup>Aa</sup>	1.30 $\pm$ 0.19 <sup>Aa</sup>
		2–4	9.9 $\pm$ 1.0 <sup>Ba</sup>	0.07 $\pm$ 0.03 <sup>Ba</sup>	8.97 $\pm$ 2.43 <sup>Aa</sup>	18.3 $\pm$ 4.9 <sup>Aa</sup>	−2.7 $\pm$ 3.9 <sup>Ba</sup>	0.19 $\pm$ 0.15 <sup>Ba</sup>
		>4	28.9 $\pm$ 2.8 <sup>Ca</sup>	0.02 $\pm$ 0.01 <sup>Ca</sup>	9.63 $\pm$ 1.24 <sup>Aa</sup>	7.8 $\pm$ 4.7 <sup>Aa</sup>	−0.16 $\pm$ 0.94 <sup>Ba</sup>	−0.06 $\pm$ 0.10 <sup>Ca</sup>
Conventional	0–10	<2	50.8 $\pm$ 3.7 <sup>Aa</sup>	0.18 $\pm$ 0.04 <sup>Ab</sup>	5.87 $\pm$ 0.35 <sup>Ab</sup>	25.1 $\pm$ 12.5 <sup>Aa</sup>	52.7 $\pm$ 23.7 <sup>Aa</sup>	1.06 $\pm$ 0.44 <sup>Ab</sup>
		2–4	10.6 $\pm$ 1.1 <sup>Ba</sup>	0.09 $\pm$ 0.03 <sup>Ba</sup>	6.60 $\pm$ 0.99 <sup>Ab</sup>	15.2 $\pm$ 7.0 <sup>ABb</sup>	21.7 $\pm$ 11.8 <sup>Ba</sup>	0.42 $\pm$ 0.35 <sup>Ba</sup>
		>4	37.7 $\pm$ 4.2 <sup>Ca</sup>	0.06 $\pm$ 0.04 <sup>Ba</sup>	8.40 $\pm$ 1.89 <sup>Bb</sup>	9.7 $\pm$ 3.8 <sup>Ba</sup>	19.5 $\pm$ 2.9 <sup>Ba</sup>	−0.09 $\pm$ 0.04 <sup>Ca</sup>
	10–20	<2	52.5 $\pm$ 8.2 <sup>Ab</sup>	0.16 $\pm$ 0.03 <sup>Aa</sup>	5.45 $\pm$ 0.43 <sup>Ab</sup>	26.6 $\pm$ 19.2 <sup>Aa</sup>	63.3 $\pm$ 26.1 <sup>Ab</sup>	0.36 $\pm$ 0.41 <sup>Ab</sup>
		2–4	9.9 $\pm$ 1.0 <sup>Ba</sup>	0.04 $\pm$ 0.02 <sup>Ba</sup>	7.14 $\pm$ 1.22 <sup>ABb</sup>	8.55 $\pm$ 4.0 <sup>Ba</sup>	24.7 $\pm$ 10.1 <sup>Bb</sup>	0.13 $\pm$ 0.12 <sup>ABa</sup>
		>4	39.5 $\pm$ 10.3 <sup>Cb</sup>	0.04 $\pm$ 0.03 <sup>Ba</sup>	7.70 $\pm$ 2.68 <sup>Bb</sup>	7.51 $\pm$ 3.3 <sup>Ba</sup>	17.0 $\pm$ 1.9 <sup>Bb</sup>	−0.06 $\pm$ 0.06 <sup>Ba</sup>

Data are the mean  $\pm$  SD ( $n = 3$ ). Different capital letters denote significant differences ( $p < 0.05$ ) among the size fractions, whereas lower case letters stand for difference ( $p < 0.05$ ) between OR and CV soils

although values tended to be higher during spring and summer (data not showed).

There were marked differences in the soil content of mineral N between D and CTO sites. Values were significantly lower ( $p < 0.01$ ) throughout the studied period in olive oil farms at CTO, especially when comparing OR orchards (Table 2).

For the whole studied period, management had not significant effect on soil mineral N, although this was dependent on site and soil sampling location (Table 3). In D site, the amount of mineral N in UTC soil position was higher under OR (values ranging between 10 and 80 kg mineral N  $\text{ha}^{-1}$ ) than under CV (1–20 kg mineral N  $\text{ha}^{-1}$ ) management throughout the studied period (Fig. 2a). This was also true for soils samples taken between tree canopy (Fig. 2c), but only for some periods (spring 2007 and summer 2008). In CTO site, soils under conventional management showed higher inorganic N content than under organically managed soils (repeated measures ANOVA), especially during late winter and early spring periods (Fig. 2b, d).

Soil mineral N in BT was generally higher than UTC (Fig. 2c). This difference was not due to a higher soil mineral N concentration in UTC position but due to the fact that the area of BT location of a hectare of olive orchards accounted for up to 75 % whereas for the UTC location it was only about 25 %.

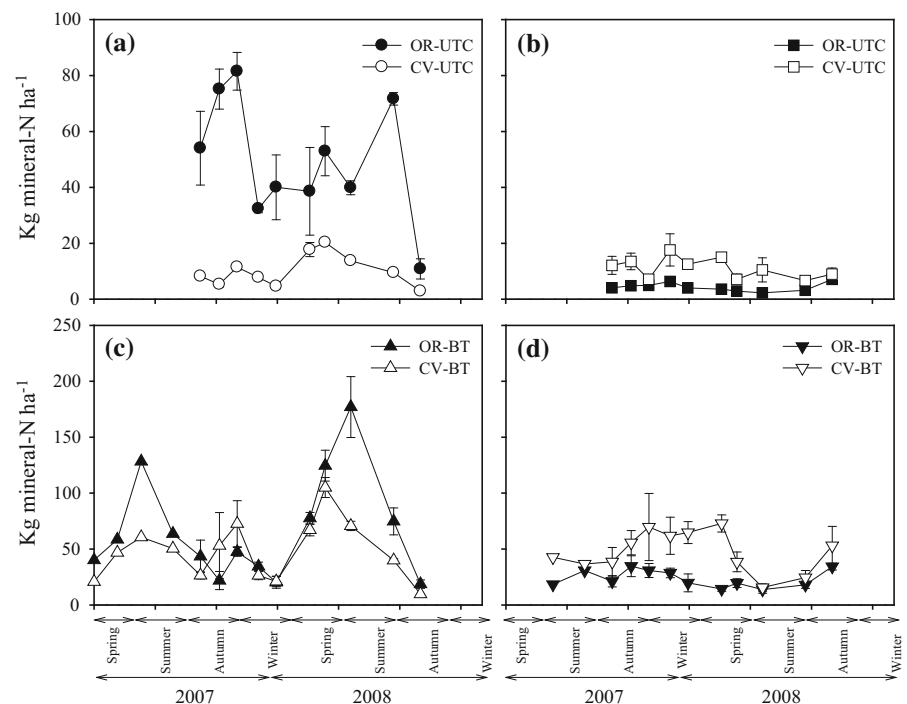
#### In situ soil NM and NN rates

Figure 3 shows the in situ soil mineral N (ammonium plus nitrate) produced by net N mineralization. In D site monthly values for both OR and CV management systems were relatively high during spring and summer (20–38 kg mineral N  $\text{ha}^{-1}$  and 75–200 kg mineral N  $\text{ha}^{-1}$  for UTC and BT, respectively). The lowest ones were recorded typically during winter, with some negative values in the CV orchards, suggesting net N immobilisation. Overall, net production of soil mineral N in CTO site was significantly lower than in D site, showing monthly values lower than 20 kg mineral N  $\text{ha}^{-1}$  in UTC soils and below 50 kg mineral N  $\text{ha}^{-1}$  in BT soil. In both soil positions and managements there were no clear temporal pattern in the soil net mineralised N. In general, values were slightly higher in late spring and summer, and peaked

**Table 3** Probability values for the effects of site, management, soil position and time on the soil available N concentration, soil available inorganic N production and net N mineralisation rate (Repeated measures ANOVA)

	Soil available N concentration	Soil available mineralN production	Net N mineralisation rate
Site (S)	<0.001	<0.001	<0.001
Management (M)	0.497	0.819	0.023
Soil position (SP)	<0.001	<0.001	0.041
S × M	<0.001	0.008	<0.001
S × SP	0.008	0.051	0.745
M × SP	0.001	0.748	0.837
Time (T)	0.006	<0.001	<0.001
T × S	<0.001	<0.001	<0.001
T × M	0.006	0.458	0.677
T × SP	0.048	<0.001	<0.001

**Fig. 2** Amount of soil mineral N (kg mineral-N ha<sup>-1</sup>) below tree canopy (UTC, **a**, **b**) and between trees (BT, **c**, **d**) for the organic (OR) and conventional (CV) olive orchards in Deifontes (D, **a**, **c**) and Cortijo Tobazo (CTO, **b**, **d**). Values are the average of 4–8 replicates and *bars* denote the SE. Note differences in scale. The area in a hectare occupied by below and between tree canopy has been taken into account for calculations

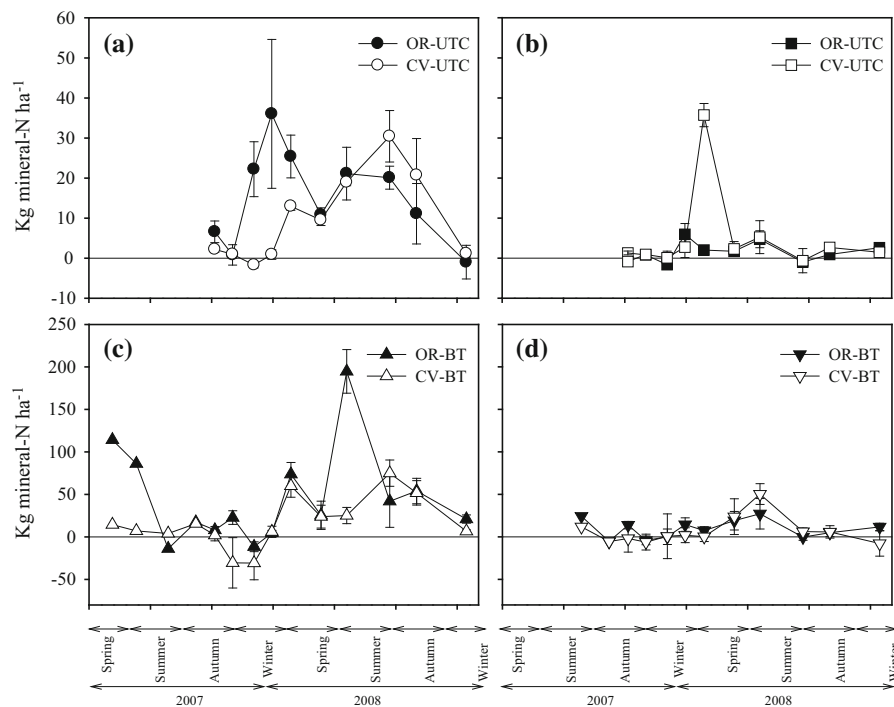


in UTC soils of CV management due to the application of urea early in this period.

Net N mineralisation rate (NM) estimated as difference of mineral N at the end and at the beginning of each period is reported in Fig. 4. Due to the generally low soil ammonium content, net N

mineralization was similar to net N nitrification (NN) rate. In general, NM rate was significantly higher in the D site in comparison to CTO site. Management had a significant effect on NM rates only in soils from D site being, overall, significantly higher in soil organically managed; but this was only true for





**Fig. 3** Monthly soil mineral N ( $\text{NO}_3^- + \text{NH}_4^+$ ) mineralised under tree canopy (UTC, **a**, **b**) and between trees (BT, **c**, **d**) for the organic (OR) and conventional (CV) management systems in Deifontes (D) and Cortijo Tobazo (CTO). Values are the

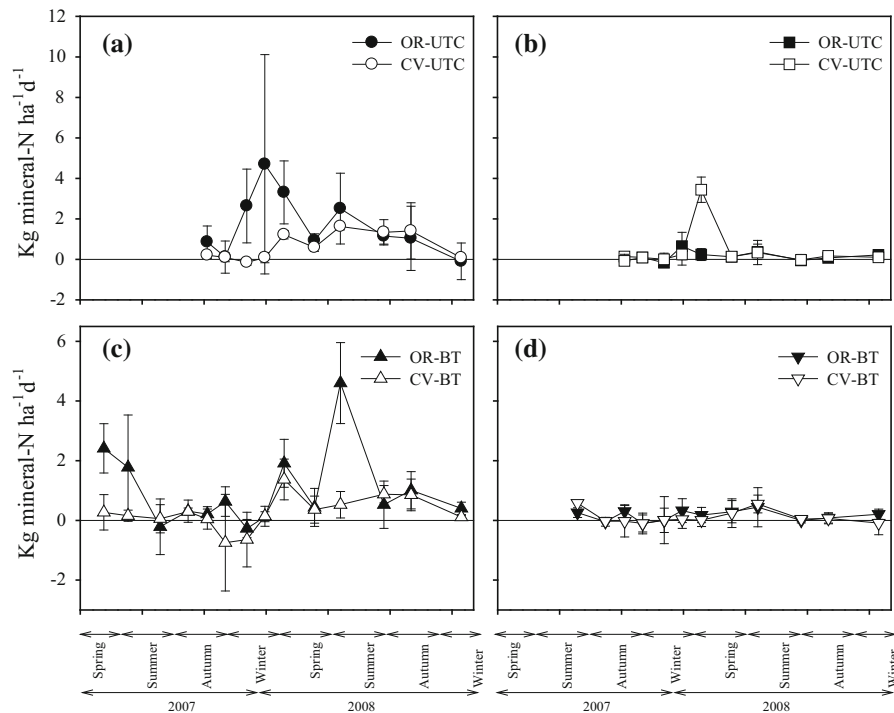
average of 4–8 replicates and bars denote the standard error. Note differences in scale. The area in a hectare occupied by below and between tree canopy and the apparent density have been taken into account for calculations

soils UTC. At D site most NM rates were positive and maxima values were found in BT position of the organically managed orchard. NM ranged  $2.6\text{--}4.7\text{ }\mu\text{g mineral N g}^{-1}\text{ day}^{-1}$  from February to April and increased up to  $4.6\text{ }\mu\text{g mineral N g}^{-1}\text{ day}^{-1}$  for UTC in OR management, during the May–June period. Minima values ( $-0.09$  to  $0.40\text{ }\mu\text{g mineral N g}^{-1}\text{ day}^{-1}$ ) were found during November–March period. In CTO site management had not significant effect on NM. The highest rate ( $3.45\text{ }\mu\text{g mineral N g}^{-1}\text{ day}^{-1}$ ) was observed in soil UTC of the conventionally managed olive oil orchards of CTO during April–March, due to fertilization with urea. However, this sharp increase lasted only 1 month.

The cumulative amount of mineral N produced during 1 year (October 2007–October 2008) at D site and for the OR management, averaged  $154\text{ kg mineral N ha}^{-1}$  for soils UTC, whereas values for soils taken between trees was  $413\text{ kg mineral N ha}^{-1}$  (Fig. 5a). Values were significantly lower in the comparable CV olive orchard which amounted 95 and  $220\text{ kg mineral N ha}^{-1}$  for UTC

and BT soils, respectively. Values in CTO site were overall much lower than in D site. Annual cumulative mineral N in the OR management system in CTO site was 14.9 and  $82\text{ kg mineral N ha}^{-1}$  for UTC and BT, respectively. For the comparable conventional farm values ranged from 48 to  $80\text{ kg mineral N ha}^{-1}$  (in the UTC and BT positions respectively) (Fig. 5b).

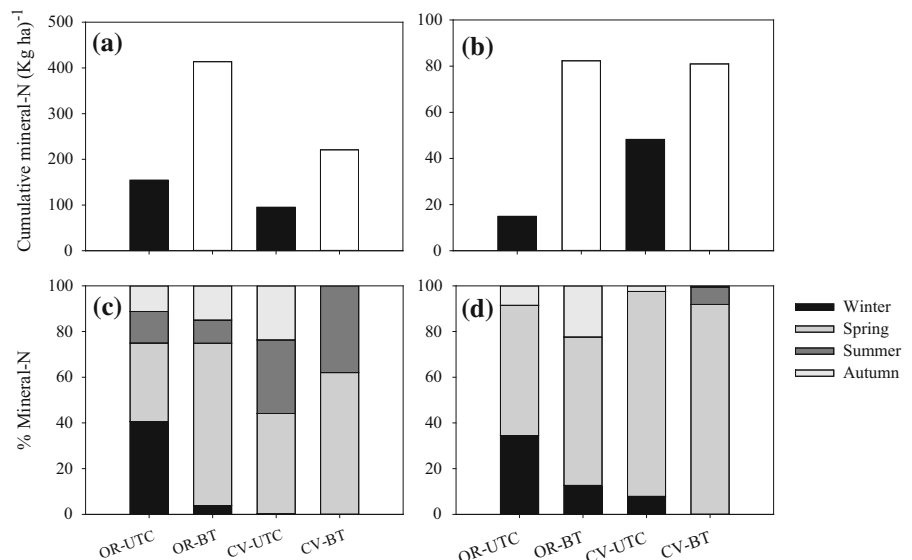
The production of available mineral N by soil was not constant during the studied period. In UTC position of the organic olive orchard in D site 40 and 34 % of the mineral N produced annually was achieved during winter and spring, respectively (Fig. 5c). However, in BT position the percentage was much lower in winter (2.8–7 %), and was much higher in spring (71 %). During summer and autumn values were intermediate (from 10 up to 14 %). In CTO site, the highest amount of mineral N was produced during spring (>60 %), especially for the conventionally managed orchards in UTC (where inorganic fertilisation was applied) (Fig. 5d). The lowest percentage of mineral N produced annually



**Fig. 4** Nitrogen mineralisation rates ( $\mu\text{g mineral-N g}^{-1} \text{ day}^{-1}$ ) in organic (OR) and conventional (CV) farming at under-tree (UTC) and between-tree (BT) at Deifontes (D, a, c) and Cortijo

Tobazo (CTO, b, d) in different sampling periods. Data are the mean  $\pm$  SD ( $n = 5$ )

**Fig. 5** Cumulative amount of mineral-N produced by N mineralisation during 1 year (a, b) and seasonal percentage of mineral-N (c, d) for all soil positions (under tree canopy and between trees, UTC and BT, respectively) and managements (organic OR and conventional CV) for D (a, c) and CTO (b, d) sites. Values are the average of 4–8 replicates



was found on summer ( $<7.6\%$ ) and intermediate values during winter (9–32 %) and autumn (–4.4 to 34.5 %), taking into account the whole set of managements and positions. No significant differences

were found between UTC and BT positions in the CV management systems.

Net N mineralisation rate was higher in soils from D site in comparison to CTO site. Soil NM rates were

also higher in OR than CV orchards (Tables 3, 4). In addition, NM rates at 0–10 cm depth were higher than at 10–20 cm, although this was significant only from January to March, April–May and September–October in D site. There were not significant differences in NM rates in the 10–20 cm between the two soil positions or the two managements, except to January–March period, when NM rates were higher in the CV management compared with their respective OR for both sites. In CTO site, there were no significant differences between the two depths (Table 4).

The net N mineralisation rates found using the bag technique were, in general, similar to those found using the intact soil-core technique, and slope of the linear relationship of values for both technique was not significantly different for a 1:1 slope ( $r^2$  0.98;  $p < 0.0001$ ). However, for spring samples, values provided by the buried-bag technique were clearly much higher than those obtained using the intact soil-core technique.

#### Correlation between some indicators of soil quality and NM and NN

PMN was positively correlated with carbonate content ( $r = 0.51$ ;  $p < 0.05$ ), sand and silt percentages ( $r = 0.60$ ;  $p < 0.05$  and  $r = 0.74$ ;  $p < 0.05$ , respectively) and TN ( $r = 0.54$ ;  $p < 0.05$ ), whereas it was negatively correlated with bulk density ( $r = -0.55$ ;  $p < 0.05$ ), exchangeable Ca ( $r = -0.42$ ;  $p < 0.05$ ), clay content ( $r = -0.67$ ;  $p < 0.05$ ) and Corg/N ratio ( $r = -0.56$ ;  $p < 0.05$ ).

The cumulative amount of nitrogen mineralised during 1 year was correlated positively with exchangeable Na ( $r = 0.52$ ;  $p < 0.05$ ), carbonate ( $r = 0.43$ ;  $p < 0.05$ ), TN ( $r = 0.52$ ;  $p < 0.05$ ), PMN ( $r = 0.68$ ;  $p < 0.05$ ), sand ( $r = 0.69$ ;  $p < 0.05$ ) and silt ( $r = 0.64$ ;  $p < 0.05$ ) and negatively with bulk density ( $r = -0.50$ ;  $p < 0.05$ ), clay ( $r = -0.56$ ;  $p < 0.05$ ) and Corg/N ( $r = -0.47$ ;  $p < 0.05$ ). In all cases, the correlations obtained for the total amount of mineral N produced were similar to those obtained for the amount of mineral N produced in each season.

There was a significant correlation between monthly mean temperature and the net amount of mineral N produced in each site ( $r = 0.35$ ;  $p < 0.05$ ). However, the soil moisture content on the samples before and after incubation was positively correlated with monthly mean temperature and rainfall for the

**Table 4** Net N mineralisation rates ( $\mu\text{g mineral-N g}^{-1}\text{d}^{-1}$ ) for organic (OR) and conventionally (CV) managed olive oil farms in 0–10 cm and 10–20 cm soil samples taken at under-tree (UTC) and between-tree (BT) area, at Deifontes (D) and Cortijo Tobazo (CTO)

Periods	Deifontes				Cortijo Tobazo			
	Organic		Conventional		Organic		Conventional	
	UTC	BT	UTC	BT	UTC	BT	UTC	BT
Jan–Mar 08	0–10	4.70 $\pm$ 5.42 <sup>a</sup>	0.54 $\pm$ 0.31 <sup>b</sup>	0.14 $\pm$ 0.34 <sup>b</sup>	0.66 $\pm$ 0.67 <sup>b</sup>	0.33 $\pm$ 0.40 <sup>b</sup>	0.25 $\pm$ 0.53 <sup>b</sup>	0.03 $\pm$ 0.30 <sup>b</sup>
Jan–Mar 08	10–20	–1.08 $\pm$ 0.75 <sup>a*</sup>	–0.42 $\pm$ 0.24 <sup>b*</sup>	–0.27 $\pm$ 0.06 <sup>b</sup>	0.73 $\pm$ 0.62 <sup>cd</sup>	0.02 $\pm$ 1.09 <sup>bc</sup>	0.96 $\pm$ 0.64 <sup>d</sup>	0.26 $\pm$ 0.31 <sup>bc</sup>
Apr–May 08	0–10	0.93 $\pm$ 0.33 <sup>a</sup>	0.36 $\pm$ 0.66 <sup>b</sup>	0.36 $\pm$ 0.45 <sup>a</sup>	0.13 $\pm$ 0.09 <sup>a</sup>	0.29 $\pm$ 0.37 <sup>a</sup>	0.14 $\pm$ 0.25 <sup>a</sup>	0.24 $\pm$ 0.48 <sup>a</sup>
Apr–May 08	10–20	0.40 $\pm$ 0.20 <sup>a*</sup>	0.25 $\pm$ 0.42 <sup>a</sup>	–0.08 $\pm$ 0.17 <sup>b</sup>	0.09 $\pm$ 0.09 <sup>a</sup>	0.40 $\pm$ 0.27 <sup>a</sup>	0.21 $\pm$ 0.25 <sup>a</sup>	0.32 $\pm$ 0.66 <sup>a</sup>
Sep–Oct 08	0–10	1.04 $\pm$ 1.59 <sup>ab</sup>	0.54 $\pm$ 0.49 <sup>abc</sup>	0.85 $\pm$ 0.53 <sup>abc</sup>	0.07 $\pm$ 0.06 <sup>c</sup>	0.08 $\pm$ 0.17 <sup>bc</sup>	0.17 $\pm$ 0.10 <sup>bc</sup>	0.06 $\pm$ 0.18 <sup>c</sup>
Sep–Oct 08	10–20	0.34 $\pm$ 1.13 <sup>a</sup>	0.20 $\pm$ 0.14 <sup>a*</sup>	0.22 $\pm$ 0.12 <sup>a</sup>	0.09 $\pm$ 0.09 <sup>a</sup>	0.40 $\pm$ 0.27 <sup>a</sup>	0.21 $\pm$ 0.25 <sup>a</sup>	0.32 $\pm$ 0.66 <sup>a</sup>
Jan–Feb 09	0–10	–0.09 $\pm$ 0.90 <sup>a</sup>	0.72 $\pm$ 0.43 <sup>b</sup>	0.11 $\pm$ 0.06 <sup>a</sup>	0.22 $\pm$ 0.18 <sup>a</sup>	0.21 $\pm$ 0.17 <sup>a</sup>	0.10 $\pm$ 0.18 <sup>a</sup>	–0.09 $\pm$ 0.39 <sup>a</sup>
Jan–Feb 09	10–20	–0.09 $\pm$ 0.60 <sup>a</sup>	0.69 $\pm$ 0.88 <sup>a</sup>	0.05 $\pm$ 0.05 <sup>a</sup>	0.24 $\pm$ 0.09 <sup>a</sup>	0.32 $\pm$ 0.19 <sup>a</sup>	0.19 $\pm$ 0.10 <sup>a</sup>	0.06 $\pm$ 0.11 <sup>a</sup>

Data are the mean  $\pm$  SD ( $n = 5$ ). Different letters stand for significant differences ( $p < 0.05$ ) in the same sampling and depths, for different position, management and sites

\* Significant differences ( $p < 0.05$ ) between depths for the same position, management and site

whole set of samples. Moreover, there was a positive correlation between the mineral N produced in each sampling and the soil water content measure after the field incubation of the bags.

The results obtained from the regression multiple between the amount of mineral N produced during 1 year and the variables measured in soil showed that total N and PMN explained 50 % of the variability of the net amount of IN produced along a hydrological cycle.

$$\text{Mineral N} = 0.217 \text{ TN} + 0.570 \text{ PMN} \quad (r^2 = 0.50)$$

## Discussion

In D site, variables related to the N availability were decreased as the depth was getting higher. This decrease was more acute in soil under OR management than for CV orchards, mainly due to the intensive tillage carried out in the CV crops. Many studies showed that a low tillage favoured higher soil organic C and N stocks, especially near the surface soils (Kern and Johnson 1993; Tan and Lal 2005). In OR orchards low-tillage or non-tillage are the more usual practices and the differences between depths are higher.

Usually the analysis of soil is carried out in <2 mm sieved soil samples. However, pools of total C and N, together with others nutrients, and C and N related microbial processes are unequally distributed among soil particles fractions (Saviozzi et al. 2007). Our results revealed that the fraction <2 mm accounted for 50–60 % of total soil, and this fraction contained the highest concentration of TN, PMN and the highest NM rates, accounting for more than 73 % of these N pools or rates. This was not unexpected as smaller soil particles have higher area-to-volume ratio and micro-aggregates and silt and clay are relatively enriched in organic matter, favouring the various soil N processes microbiology mediated.

Soil mineral N and in situ net N mineralisation and nitrification

Overall soil ammonium content was very low and closed to zero resulting in similar NM and NN values. This was not unexpected since soil ammonium concentration is usually low and with residence time as short as less 5 days (Murphy et al. 1998) in

agricultural soil, mainly because  $\text{NH}_4^+$  is rapidly nitrified (Robertson 1997) or immobilized (Azam et al. 1993).

A high variability in soil mineral N concentration and in situ net N mineralisation and nitrification was found between sites, management practices within a site and within different locations in the same orchard. Overall, soil mineral N levels in the organic orchard located in D were higher than the conventionally managed ones. Values for both types of management were similar to those reported by Ma et al. (1999) and Booth et al. (2005) for different agricultural soils. In CTO, however, soils of the conventionally managed orchard showed higher levels of mineral N than the organic farm only during spring sampling, and this was due to the early spring application of N readily available chemical fertilisers in the conventional olive oil farm.

NM and NN rates of this study were estimated using the buried-bag technique. According to Hanselman et al. (2004) there is uncertainty regarding the extrapolation of the results obtained by this method in field because the N mineralisation process can be affected by a number of dynamic and site-specific factors (e.g. fluctuating temperature, water, aeration). However, Hanselman et al. (2004) showed that results of buried-bag techniques were similar to that of laboratory incubation, but both overestimated the long-term in situ NM. However, in short term studies (<45 days), like ours, this technique was proved to be adequate for the estimation of NM. Monaco et al. (2010) also found that the buried-bag technique provides a consistent assessment of the dynamic of NM. In this study, the NM and NN values calculated with the buried-bag technique were similar of that soil cores, and they were not found to be significantly differences from a 1:1 lineal model. However, there was a trend towards overestimation of the NM using buried-bag technique and therefore caution should be taken in comparative studies, especially in spring.

The rate of soil N mineralisation is controlled by large number factors in a complex manner (Benbi and Richter 2002). Despite the intricate controls on this process, and the involvement of a vast array of microbes of different taxonomic groups, the rate of mineralisation is largely dependent on the quantity and quality (composition) of organic matter and the influence of environmental factors on biological activity (Goncalves and Cerlyle 1994). NM and NN

were higher in the soil of D, which also showed higher TN and PMN content; both variables have been early considered proxy indexes of N availability (Griffin and Laine 1983; Serna and Pomares 1991). Due to the many variables involved in the control of both NM and NN, it is difficult to find a single explanation to the differences observed when comparing between sites differing in soil properties and environmental conditions together with management type. Nevertheless, the lower TN content in CTO soils, together with the relatively high soil clay content, might explain the overall lower rate of NM and NN at this site. Sainju et al. (2002) observed that an increase in soil clay content of only 5 % produced a significant reduction in the mineralisation rate of organic carbon and N.

Overall and throughout the year, NM in the OR managed orchards were higher than in CV ones. In our case, selected study plots were comparable in terms of environmental condition and soil properties, thus differences in NM are related mainly to management practices. OR olive orchards allows for natural plant growing in the intercanopy area from May to the following February, and the presence of this vegetation might increase NM because: (1) plant residues improve soil fertility indicators such as soil organic matter and carbon, cation exchange capacity, soil water content favoured due to higher rainfall infiltration, (2) natural plant uptake of residual organic N fertiliser might limits N losses by leaching and increase the pool of TN and PMN. Sainju et al. (2000) and Gómez-Muñoz et al. (2014) have shown that the presence of cover crops increases the reutilization of residual inorganic and organic N fertiliser. The increase of soil organic C and N following incorporation of plant-cover depends on the amount and quality of residues, rate and mechanism of application, soil type, tillage regime and climatic condition (Stevenson 1982). Thus, all these factors might play an important role in the differences in the NM between organic olive orchards in D and CTO sites. In D site natural plant biomass productivity was more than three times higher than that found in CTO site (data not shown) and it might explain some of the differences in the NM, whereas in CV orchards of D and CTO natural vegetation were absent because they were controlled by a combination of both herbicides and tillage. It has been shown that low tillage enhances the mineralisation of soil organic C and N by incorporating plant residues, disrupting soil aggregates, increasing aeration, and altering soil

temperature and moisture that favour microbial degradation of organic matter (Balesdent et al. 1990; Dalal and Mayer 1986; Cambardella and Elliot 1993). However, to achieve these improvements on soil by tillage, this has to be done at a low frequency and intensity (Franzluebbers et al. 1995). The low tillage regime performed in D not only mixed the natural plant residues with the soil but also increased decomposition. However, at CTO where tillage was not practiced, decomposition of natural plant residues is expected to be slow.

NM rates were slightly higher in soils UTC than in soil between trees (BT), and this was true in both sites (D and CTO) and for the two management practices (OR and CV). Environmental conditions in UTC (higher soil water content and more constant temperature) were more favourable for N mineralisation and nitrification than in BT position, explaining this fact.

Overall, NM decreased with depth reaching negative values, especially for some samples in the OR management system. Similar results were found by Cambardella and Elliot (1992), for other grassland soils. The higher values of NM in the first cm of soil were not unexpected since the top 10 cm soils contain highest values of short-term (PMN) and medium-to-long term N available (TN). However, in the CV soils, NM was not significantly different between the two depths, likely because tillage made more homogeneous the first 20 cm of soil together with an increase in the oxygen diffusion (Bayer et al. 2001).

Previous studies on soil N mineralisation demonstrated that marked seasonal and temporal variation can occur in different ecosystems, such as grasslands (Steltzer and Bowman 1998), forest (Vitousek and Matson 1985) and other ecosystems (Schimel et al. 2004). During the growing season due to a higher temperature couple to a greater soil moisture (Zhu and Carreiro 2004) and non-growing season due to microorganisms immobilizing nutrients during summer nutrients are released from lysed cells of dying microbes during winter (Schimel et al. 2004). In our case, the highest rates of N mineralisation were found during the growing season. The highest amount of mineralised and nitrified N was found also in spring in both sites, when high temperatures and medium level of rainfall coincided. In winter, on the other hand, the percentage of N produced was near zero or even negative at both sites, suggesting some N immobilization, especially in the conventionally managed olive orchards.

In contrast, the amount of mineral N produced during winter in OR management system, especially in UTC position, was close to 40 % of total N produced. The main differences in the percentages between the two sites were found in summer, where the percentage was around 20 % in D, likely due to a higher microbial activity as a consequence of irrigation.

During a hydrological cycle, the net mineral N produced in D by NM and NN in the BT soils of the OR orchards was  $413 \text{ kg ha}^{-1}$ , whereas values were almost halved ( $220 \text{ kg ha}^{-1}$ ) in the comparable CV orchard. In CTO site, however, values were much lower ( $82$  and  $80 \text{ kg ha}^{-1}$  for BT from OR and CV orchards, respectively). In all the cases, the amount of N produced by the soil was much higher than that demanded for the crop which is estimated around  $35 \text{ kg N ha}^{-1}$  (Fernández-Escobar et al. 2012), though it is highly dependent on production. Annual rate of application of N fertiliser, typically in a single dose during late winter, reported for olive orchards is around  $9\text{--}350 \text{ kg N ha}^{-1}$  (Fernández-Escobar et al. 2006). However, the olive tree N uptake is not constant along the year. Highest demand takes place in March–June and in October (García-Ruiz et al. 2011). The high amount of soil inorganic N found in the CV orchard in both sites during the April–May period coincided when inorganic fertiliser were applied. However, in the following sampling after N fertiliser was applied, the content of soil mineral N was relatively low, suggesting the occurrence of high N immobilization, leaching and/or denitrification. For OR olive orchard in D site, the supply of soil mineral N by NM and NN from the organic N pool occurred during March up to November, which could be able to satisfy the requirement of the olive trees. However, for OR farming in CTO site the amount soil mineral N supply was much lower, likely due to the low-medium quality of soil and low natural plant biomass production. Hence in this case an extra N organic fertilisation would be necessary to match soil mineral N supply with the N demand of trees.

#### Correlation between general soil properties and soil NM and NN

PMN is well known as good indicator of N availability in soil. In this study, PMN was positively correlated with TC and TN among other variables. The

relationship between these two variables and PMN has already been reported (Hassink 1995). PMN on the other hand, was negatively correlated with bulk density and clay content. A high soil clay content might prevent the decomposition of organic substrates by increasing their chemical and physical protection (Yoo and Wander 2006). Delin and Lindén (2002) found that soil organic matter and clay content explained 23 % of the within-field variation in net soil N accumulation during the growing season. Alternatively, Thomsen et al. (2001) found no direct soil textural effects on N mineralisation. However, higher microbial activity has been reported in coarse—than in fine—textured soils (Hassink 1994; Franzluebbers et al. 1996). In spring and summer periods, characterized by high temperature and low-moderated rainfall, NM and NN rates were correlated positively with the percentage of sand and silt in soil, which might be closely related to soil water retention.

NM, NN and the amount of mineral N produced by soil were, for most sampling times, positively correlated with the content of TN and PMN, but negatively with the organic C:N ratio. These relationships are well documented and highlight the great impacts of TN on the net N fluxes at the short-term (Booth et al. 2005).

Campbell and Biederbeck (1972) reported that soil microclimate also plays a very important role in regulating N availability and the rate of soil N transformation, including soil N mineralisation, which are mainly microbiologically controlled. The main controlling factors of NM and NN are temperature, water content, and soil architecture (McGill and Myers 1987), the latter through its effect on the pore-size distribution and soil aeration. In our study, soil moisture content before and after incubation was correlated with mineral N produced, NM and NN, highlighting the important role of water content in these rates. Similar relations were reported by Paul et al. (2003) who proposed that the maximum N mineralisation rate use to occur when the soil water content is near to the water holding capacity.

Campbell et al. (2008) found that precipitation (measured between spring and autumn during a 40 yr period on an experimental site in Saskatchewan, Canada) accounted for between 12 and 43 % of the variability in net N mineralised during the growing season depending on the crop rotation. Davidson and Janssens (2006) showed the marked effects of temperature on soil NM. Moreover, according to Ellert



and Bettany (1992) the dependence of N mineralisation on temperature may be more important than the amount of potentially mineralisable N presents in the soil in determining the plant availability of N during a growing season. Kirschbaum (1995) reported that temperature influences over decomposition, measured as soil respiration, which increased with decreasing soil temperature; whereas soil N mineralisation appeared to exhibit less temperature sensitivity compared with decomposition. This influence could not be tested in our experiment due to the low differences in environmental condition in both site of study, which had similar rainfall and temperature.

Finally, the results obtained from the multiple regression analysis between the amount of soil mineral N produced during 1 year and the measured variables indicates that the TN and PMN were able to predict the soil mineral N produced during one hydrological cycle ( $r^2 = 0.50$ ). Similar findings have been described by several authors as Constantinides and Fownes (1994) or Jensen et al. (2005).

## Conclusions

The magnitude and the temporal patterns of soil available N were controlled by soil properties and environmental conditions, both modified by management practices in olive oil orchards. In olive orchards from D site, soil N mineralisation and nitrification were higher than from CTO site, likely were higher than from CTO site, likely the lower soil clay content of the former. Location of the soils within the olive oil orchard was also found to be an important source of variation of the net N which was mineralised and nitrified. Management system also influenced MN and NN. In D site, soil N mineralisation was higher in organically managed orchard than in the conventionally managed one. In contrast, in CTO site, the soil N mineralisation was higher for CV, due to the high rates of chemical fertilisers applied in this orchard. Total N and the pool of PMN explained together 50 % of the variability in the soil N availability over a year, suggesting that these soil properties were good predictors of the potential for a soil to provide available N at yearly basis. The highest rates of soil available N (measured as net N mineralisation and nitrification) were found in spring (up to 80 % of the annual amount), when olive tree demand for N was at

its maximum. Annual soil available N in olive groves was in all cases higher or similar than tree demand suggesting that the annual supply of N by the soil should be taking into account when developing N fertilisation strategies for olive crops.

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