Influence of Sampling Date on Soil Nitrogen Availability Indices

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*In spite of the great effort that has been devoted to the search for a chemical laboratory index to predict nitrogen (N) mineralization capability of soils, the results have not yet been fully satisfactory. A continued effort is still needed to increase the knowledge of the sources of variation that influence potentially available soil N. The time of sampling has received little attention, taking into account its potential to influence N-mineralization patterns. In this work, soil samples from three different agrosystems, consisting of a double-crop sequence of small grains and maize, an intensively grazed pasture, and a rainfed olive orchard, were collected at different dates. Several chemical extractions were performed, and the results were correlated with N uptake by turnip (*Brassica campestris*, L.) grown in a pot experiment. Kjeldahl N was the chemical test that best correlated ($R^2 = 0.621$) with N uptake by turnip. Kjeldahl N showed great versatility relative to the origin of the soil samples. However, it was not very sensitive to the time of sampling. It did not detect changes occurring in the soil over a short period of time. Soil inorganic N showed the second highest coefficient of correlation ($R^2 = 0.483$) with N uptake by turnip. In contrast to that observed with Kjeldahl N, soil inorganic N appeared as an index that can vary greatly over the short term. The hot saline potassium chloride (KCl) extractions gave generally fair results. The poorest, however, were obtained with the ultraviolet absorption of extracts of 0.01 mol L^{-1} sodium bicarbonate ($NaHCO_3$) measured at 250- and 260-nm wavelengths.*

Keywords Hot KCl-NH_4, Kjeldahl N, $NaHCO_3$ UV absorption, nitrogen availability indices, organic C, pot experiment, soil inorganic N

Introduction

Since the 1950s a great deal of scientific effort has been devoted to the development of an empirical chemical procedure to predict the nitrogen (N) mineralization capability of a soil that could be used routinely as the basis for N-recommendation programs (Harmsen and van Schreven 1955; Keeney and Bremner 1966; Stanford and Smith 1976). Many chemical extractants have been investigated (acids, alkalis, salts, water, etc.) as possible tools for assessing how much N may be mineralized from a soil during a growing season, with variation in the concentration of the extracting agent, the temperature, and the time of extractions. The results of the chemical methods were related to biological indices, such as mineralizable N in laboratory aerobic and/or anaerobic incubations (Smith and Stanford 1971; Gianello and Bremner 1986; Jalil et al. 1996; Wang et al. 2001; Sharifi

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et al. 2007), N recovered in pot experiments (Robinson 1968; Serna and Pomares 1992; Smith and Li 1993), or indices of soil N availability obtained from field-grown crops (Fox and Piekielek 1978a, 1978b; Hong, Fox, and Piekielek 1990; McTaggart and Smith 1993; Paul and Beauchamp 1993).

During the 1980s and 1990s, the published work continued to be dominated by great optimism, with the researchers usually finding good relationships between the chemical and biological indices (Gianello and Bremner 1986; McTaggart and Smith 1993; Smith and Li 1993; Jalil et al. 1996). However, after decades of searching for an easy-to-use chemical index to estimate the N-mineralization capacity of a soil, there is no report that any have been included in a concrete N-recommendation program. Many conflicting results have been appearing in the literature after a general inability by researchers to replicate the positive results already found. Particular difficulties have been experienced when the biological indices were obtained from crops grown under field conditions (Hong, Fox, and Piekielek 1990; Walley et al. 2002).

Most of the previous work has been carried out on soil samples provided from a wide range of climatic zones and soil types (Smith and Stanford 1971; Stanford and Smith 1976; Gianello and Bremner 1986; Jalil et al. 1996; Wang et al. 2001; Sharifi et al. 2007). Some authors restricted their studies to a given crop or a particular agrosystem (Hong, Fox, and Piekielek 1990; McTaggart and Smith 1993; Smith and Li 1993). Others included soils either previously managed under different tillage systems, or with legumes as a preceding crop, or with additions of farmyard manure or lime (Beauchamp, Pararajasingham, and Kay 2003; Soon, Haq, and Arshad 2007; Sharifi et al. 2008). Spatial variability, as a potential problem in predicting the quantity of N mineralized under field conditions, has already been studied by Mahmoudjafari et al. (1997) and Zubillaga, Cabrera, and Vaio (2009). In addition, the methodologies applied to the soil samples have also been tested to predict the N mineralized from organic substrates, such as manures and sewage sludge (Serna and Pomares 1992; Cordovil et al. 2007).

It was pointed out in an earlier work by Robinson (1968) that the time of the year or season at which the soil samples are collected from the field affects mineralizable N. It would be expected, therefore, that the most labile fraction of organic N, which plays a prominent role as a source of substrate for N mineralization, varies over the year as a result of the deposition of debris of crops and weeds and root death. However, studies where the time of sampling has been tested as a source of variation are practically nonexistent. In this work, the effect of sampling date on soil N availability was investigated in three substantially different agrosystems: (i) an intensive double cropping system, consisting of a mixture of small grains (October to May) and maize (May to September) with both crops grown for silage; (ii) an intensively grazed pasture dominated by legume species; and (iii) and a rainfed olive orchard, which was divided into two plots in 2001, one of which was managed by conventional tillage and the other with a nonselective herbicide. Several chemical tests were performed and their results were compared to N uptake by turnip (*Brassica campestris* L.) grown in a pot experiment. The performance of each chemical index was evaluated, taking into account differences in the soil samples not only between the different agrosystems but also within each agrosystem.

Materials and Methods

Agrosystems and Soil Sampling

The soil samples used in this study were taken from three completely different agrosystems in the north of Portugal: (i) an intensive cropping system based on small grains and maize crops, both grown for silage; (ii) an intensively grazed pasture; and (iii) a rainfed olive orchard.

The field where the intensive crop rotation of small grains and maize was conducted is located near the city of Bragança (41° 48′ N; 6° 44′ W). The region benefits from a Mediterranean climate with some Atlantic influence. Mean annual temperature and annual precipitation are 11.9 °C and 741 mm. The small grains and maize crops were grown in a double-cropping sequence where both crops were cultivated in the same field in each year, with the small grains crop from October to May and maize from May to September. The small grain crop was composed of a mixture of ryegrass (*Lolium multiflorum* L.), triticale (*X Triticosecale* Witt.), oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.). This crop sequence is repeated over the years, acting as a monocultural system. The seedbed was prepared by plowing and scarifying the soil in a similar manner for both crops, respectively, in October and May for small grains and maize crops. Maize, which was grown in the warm season, was sprinkle irrigated. The small grains were fertilized as topdress, applying a rate of 60 kg N ha^{-1}. The maize was fertilized at preplant and as topdress by using 60 + 60 kg N ha^{-1}. Soil samples were collected on 15 October 2008 and 22 May 2009 from a depth of 0–20 cm. For each sampling date four replications were collected. Each composite sample was prepared from 10 subsamples. The soil samples were oven dried at 40 °C in thin layers in trays and passed through a 2-mm sieve. The soil is a Eutric Cambisol sandy loam. The size analysis revealed a soil of 66% sand, 18% silt, and 16% clay. Other soil properties based on the sampling date of October are presented in Table 1. Soil organic C and total soil N are not shown in the Table 1 because they were used as N-mineralization indices and are presented as results.

The intensively grazed pasture is located near the city of Covilhã (40° 16′ N; 7° 30′ W). Mean annual temperature and annual precipitation in the region are 13.9 °C and 1034 mm. The soil is a Leptosol derived from granites with a sandy loam texture. The soil separates were 75% sand, 12% silt, and 13% clay. Some chemical soil properties are presented in Table 1. The sward was sown in October 2001 from a mixture of up to 20 different species of grasses and legumes. In spring 2008 the pasture was dominated by legume species, in particular *Trifolium subterraneum*, with a mean groundcover of more than 80%. Other common species were *T. cernuun*, *T. glomeratum*, *T. dubium*, *Agrostis salmantica*, *Plantago coronopus*, *Leontodon longirostris*, and *Chamaemelum mixtum*. The intensively grazed pasture received 1500 kg lime ha^{-1} at the time of sowing. Thereafter, a rate of 150 kg ha^{-1} of superphosphate 18% was biennially applied. The pasture is continuously grazed over almost all the year by a mixture of cattle and sheep with a total stocking rate estimated in 1.0 livestock units. Grazing only stops from early April to late May to allow flowering and grain filling of the self-reseeding annual species. The soil was sampled at a depth of 0–20 cm at 10 times over the period of a year, from October 2007 to October 2008. For each sampling date four replications were collected at each field site. Each composite sample was prepared from 10 subsamples.

The olive orchard is also located near the city of Bragança. The orchard is planted in a Regosol, loam textured, consisting of 50% sand, 34% silt, and 16% clay. The orchard is approximately 60 years old, with 200 trees ha^{-1} of the cultivar Cobrançosa. It has been managed since 2001 under two different groundcover treatments: tillage, consisting of two tillage passes per year in spring by cultivator, and postemergence herbicide (glyphosate, 360 g L^{-1} of active ingredient) applied yearly in the first fortnight of April. Before 2001, the orchard was managed as a sheep walk. The fertilizer regime included the annual application of a compound 10:10:10 [10% N, phosphorus pentoxide (P_2O_5), dipotassium oxide (K_2O)] fertilizer and borax (11% boron) at rates of 1500 and 70 g $tree^{-1}$, respectively. The orchard was pruned triennially in March 2003 and 2006. Soil samples were collected at a

Table 1

Selected properties of soils based on the first date of sampling of each agrosystem [small grains and maize crops (SGM); intensively grazed pasture (IGP); and rainfed olive orchard]

			Rainfed olive orchard			
			Tillage		Glyphosate	
Soil properties	SGM	IGP	B. trees	B. rows	B. trees	B. rows
pH (soil:water, 1:2.5)	7.0	5.9	5.7	5.7	5.7	5.6
Extractable P (Egner-Rhiem) (mg kg^{-1})[a]	174.5	67.5	56.3	26.7	159.8	36.4
Extractable K (Egner-Rhiem) (mg kg^{-1})[a]	157.7	240.6	119.5	101.2	183.4	112.4
Exchangeable bases (ammonium acetate, pH 7)						
Ca ($cmol_c$ kg^{-1})	10.78	3.98	8.39	7.55	7.31	7.02
Mg ($cmol_c$ kg^{-1})	4.88	0.80	2.38	2.09	2.03	2.04
K ($cmol_c$ kg^{-1})	0.88	0.39	0.57	0.42	1.15	0.68
Na ($cmol_c$ kg^{-1})	0.78	0.70	0.66	0.68	0.70	0.69
Exchangeable acidity ($cmol_c$ kg^{-1})	0.25	0.35	0.35	0.35	0.15	0.35
Cation Exchange capacity ($cmol_c$ kg^{-1})	17.57	6.22	12.34	11.07	11.32	10.77

[a]Extracted by ammonium lactate plus acetic acid, buffered at pH 3.7.

depth of 0–20 cm, beneath the tree canopies and between the rows in both soil management systems. Soil was also sampled at two different dates, in September 2008 after the first rains and in May 2009. The soil sampling procedures were similar to those described for the other agrosystems. The soil pH and other chemical soil properties are presented in Table 1.

Chemical Extraction Methods

The chemical extraction methods used in this work were essentially similar to some of those reported by Sharifi et al. (2007) and Soon, Haq, and Arshad (2007). Extractable ammonium (NH_4)-N plus nitrate (NO_3) N [potassium chloride (KCl) Nmin] were determined from 10.0 g of soil and 40 mL 2 M KCl shaken for 1 h. The suspension was thereafter filtered through a Whatman No. 42 filter paper. The concentrations of NH_4-N and NO_3-N in the extracts were determined by ultraviolet (UV)–visible spectrophotometry. Hot KCl–extractable NH_4-N (HKCl-NH_4) was determined by heating (100 °C) 5.0 g of soil and 20 mL of 2M KCl for 4 h in a digestion block. Thereafter the tubes were removed and allowed to cool, and the extract was filtered through a Whatman No. 42. Hydrolysable NH_4-N (Hyd NH_4) was obtained by subtracting initial NH_4-N from hot KCl–extractable NH_4-N. The UV absorbance of sodium bicarbonate ($NaHCO_3$) extracts were prepared by shaking 2.5 g soil with 0.01 mol L^{-1} $NaHCO_3$ for 15 min. The suspension was filtered through a Whatman No. 42 paper. The UV absorbance of the extracts was measured at 205 ($NaHCO_3$-205) and 260 ($NaHCO_3$-260) nm wavelengths. Kjeldahl N (Kjel N) was determined after the digestion of 1.0 g of soil with H_2SO_4 and selenium as the catalytic agent in a heated (400 °C) aluminum digestion block. After cooling, the suspension was distilled with alkali, and the NH_4-N in the digest was titrated with hydrochloric acid (HCl) in a Kjeltec Auto 1030 Analyzer (Tecator, Sweeden). Oxidizable organic carbon (organic C) was determined by wet oxidation in an acid dichromate solution followed by back titration of the remaining dichromate with ferrous ammonium sulfate and phenanthroline as the color indicator.

Pot Experiment

Turnip (*Brassica campestris* L.) was the test crop used in a pot experiment to determine plant N uptake as a biological index of soil available N because of its nitrophily and fast growth. The soil samples were air dried and sieved (2-mm mesh). Four pots (replications) per treatment were filled with 1.0 kg dry soil. Ten seeds of turnip per pot were sown on 1 February 2009, followed by the first irrigation with 150 mL of distilled water. After emergence, the plants were thinned to five plants per pot. Thereafter, the soil was regularly watered (two to four times per week) with 75 mL of distilled water. The number of irrigations varied with the growth stage of the crop and the different treatments, taking into account the influence of those factors on the transpiration rate of the turnip plants. Dishes under the pots prevented water loss and nutrient leaching. The small amounts of water applied at each irrigation were intended to reduce nitrate denitrification. When the plants stopped growing because of the exhaustion of available nutrients, they were cut and the aboveground biomass was oven dried at 70 °C, weighed, and ground. Total N content in dry material was determined in a Kjeltec 1030 Auto analyzer (Tecator, Sweeden).

Data Analysis

A correlation analysis was established between the chemical N-mineralization indices and N uptake by turnip in the pot experiment. The effect of the sampling date on N-mineralization indices was evaluated by analysis of variance (ANOVA). After ANOVA examination, the means with significant differences ($\alpha < 0.05$) were separated by the HSD test.

Results and Discussion

Relationship between N-Mineralization Indices and N Uptake by Turnip

Almost all the N-mineralization indices were linearly related to N uptake by turnip grown in the pot experiment. The greatest coefficient of determination ($R^2 = 0.621$) was found from the relationship between Kjeldahl N and N uptake by turnip (Figure 1a). Soil organic C was also significantly linearly related ($R^2 = 0.224$) to N uptake by turnip (Figure 1d).

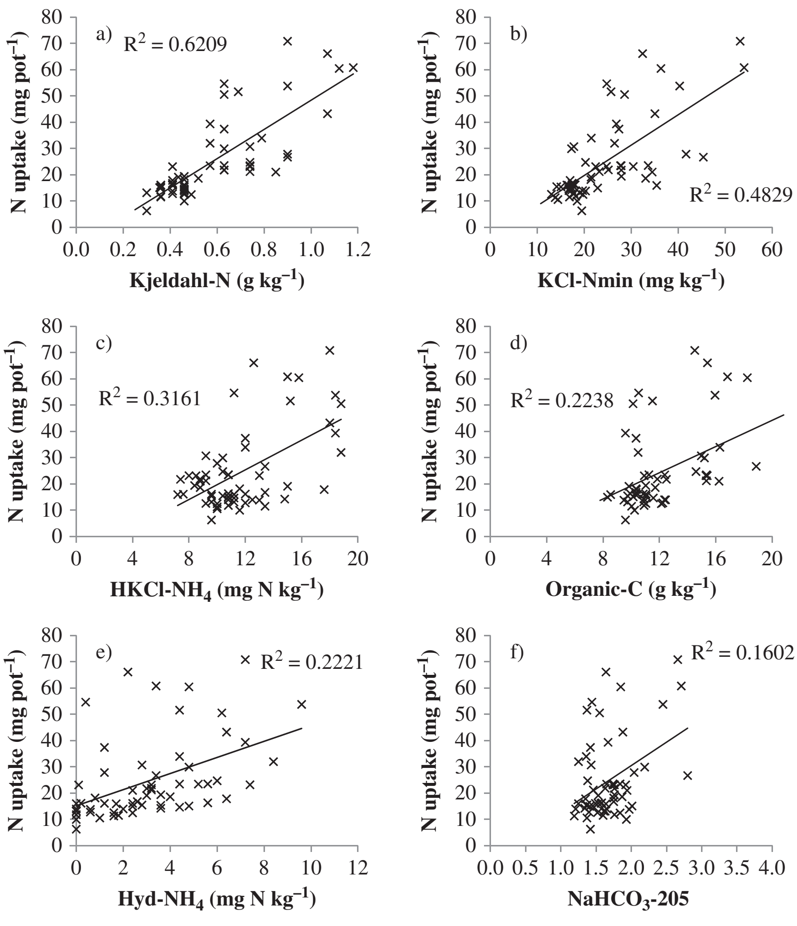

Figure 1. Relationship between chemical N mineralization indices and N uptake by turnip in the pot experiment.

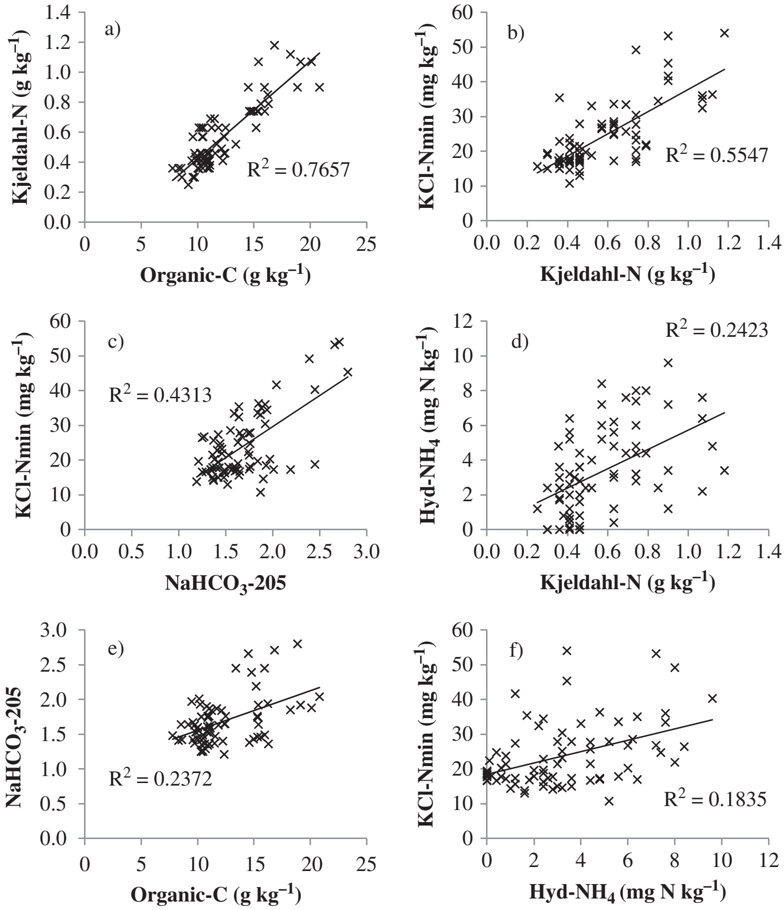

Figure 2. Relationships between the chemical N mineralization indices.

Both Kjeldahl N and organic C represent the total soil organic matter, the former usually representing 5% of the latter (Boswell, Meisinger, and Case 1985). Thus, the close correlation ($R^2 = 0.766$) between Kjeldahl N and organic C (Figure 2a) was not surprising. There have been studies where good correlations were found between organic N and biological indices of mineralizable N (McTaggart and Smith 1993; Wang et al. 2001). Soil organic matter has also been the only index of N-supply power of soils widely used in N-advisory systems (Boswell, Meisinger, and Case 1985). In several other studies, however, contradictory results have been found, with soil organic matter (or total organic N) being a poor predictor of potentially available N (Smith and Stanford 1971; Fox and Piekielek 1978b; Gianello and Bremner 1986; Hong, Fox, and Piekielek 1990; Scott, Norris, and Burger 2005). In this study, the close correlation established between Kjeldahl N and N uptake by turnip was possibly due in part to the artificiality of the pot experiment, where the intense aeration (by soil sieving), soil moisture (by plant irrigation), and temperature favored the microbial attack of all the organic-matter fractions, leading to a net N mineralization measured by N uptake by turnip, directly related to the total soil organic N.

The correlation between KCl Nmin and N uptake by turnip can also be considered as high ($R^2 = 0.483$) (Figure 1b). Kjeldahl N and KCl Nmin, which have been the two best predictors of N mineralization in this study, appeared also to be closely correlated ($R^2 = 0.555$) with each other (Figure 2b). Soil inorganic N has frequently been found as a good indicator of the N-supply power of soils. Giles, Reuss, and Ludwick (1975) reported that the soil NO_3-N levels measured before planting were useful in estimating the N requirements of a sugarbeet crop. Close correlations between soil inorganic N and biological indices were also found by Roberts, Weaver, and Phelps (1980) and Paul and Beauchamp (1993). It seems that the inorganic N in the soil profile not only represents a quantitative value but is also a qualitative index, providing a good indication of the N-mineralization potential of a soil (Giles, Reuss, and Ludwick 1975; Roberts, Weaver, and Phelps 1980). The soil NO_3-N in the soil profile before sowing has often been used as the basis for fertilizer N recommendations, in particular in the advisory systems of semi-arid regions (Boswell, Meisinger, and Case 1985). Significant success has also been the preside dress soil nitrate test (PSNT) introduced by Magdoff, Ross, and Amadon (1984), a soil test for N availability in corn. It consists of the determination of soil NO_3-N level prior of the time for sidedress fertilizer application as the basis for fertilizer N recommendations. Many other researchers reported that PSNT successfully distinguished N-sufficient from N-insufficient sites (Hong, Fox, and Piekielek 1990, Heckman et al. 1996) particularly when ($NO_3 + NH_4$) N was used instead of NO_3-N alone (Meisinger et al. 1992).

The correlation between HKCl-NH_4 and N uptake by turnip showed the third greatest coefficient of determination ($R^2 = 0.316$) among the chemical indices (Figure 1c). Hyd NH_4 was more poorly correlated ($R^2 = 0.222$) to N uptake by turnip than HKCl-NH_4 (Figure 1e). The correlation between HKCl-NH_4 and Hyd NH_4 and between these indices and the other chemical extractants was weak or nonexistent (Figure 2). The saline hot KCl extractions have been reported as the most promising indices of N mineralization, because good correlations with the biological tests have usually been found (Gianello and Bremner 1986; McTaggart and Smith 1993; Smith and Li 1993; Cordovil et al. 2007; Sharifi et al. 2007). In the present study, Hyd NH_4 gave an even poorer correlation with N uptake by turnip than HKCl NH_4. Jalil et al. (1996) also observed that when NH_4-N extracted with cold 2 M KCl was subtracted from the NH_4-N extracted with hot 2 M KCL solutions, the association with N mineralized by aerobic incubation was weaker. The hot KCl solutions have not always given positive results. Walley et al. (2002) observed that hot KCl extract did not explain more variability in crop N accumulation than did basic soil properties. According to the results of Hong, Fox, and Piekielek (1990) and Scott, Norris, and Burger (2005), neither are the saline KCl solutions useful for estimating mineralizable N in soils.

The $NaHCO_3$-205 was poorly correlated to N uptake by turnip (Figure 1f), whereas $NaHCO_3$-260 was not even significantly related to the biological index. In other studies, however, the UV absorption by 0.01 mol L^{-1} $NaHCO_3$ soil extractant at 260 nm and/or 205 nm was found to be well correlated with the N-supply capability of soils (Fox and Piekielek 1978a; Sharifi et al. 2007). The $NaHCO_3$-205 measures both organic and mineral forms of N, whereas the $NaHCO_3$-260 only measures organic N forms in the extract (Fox and Piekielek 1978a; Sharifi et al. 2007). This may explain the close correlation of $NaHCO_3$-205 with KCl Nmin found in this study (Figure 2c). Because KCl Nmin was highly correlated with N uptake by turnip, this may also justify the relatively better performance of the $NaHCO_3$-205 index in comparison to $NaHCO_3$-260.

Effect of Sampling Date on N-Mineralization Indices

From the soil samples collected in May, in the field where the small grains and maize were grown, the N uptake by turnip was 31.53 mg pot^{-1}. The N uptake from the soil samples collected in October was 23.03 mg pot^{-1}, and the difference between the two means is statistically significant (Table 2). In contrast, soil inorganic N levels were significantly lower in soil samples taken up in May in comparison with those collected in October. In May, the soil was sampled shortly after the cut of the small grains crop. Thus, the soil inorganic N levels were lower because the plants were taking up nutrients right up to the moment when they were cut and the soil was sampled. In October, the period between the cut of the maize and soil sampling was longer, which allowed the accumulation of more inorganic N in the soil through the mineralization of the organic substrates. Nitrogen uptake by turnip in turn reflects the amount of N mineralized over a period of several months, in the course of the pot experiment. Net N mineralization was greater following the small grains crop than maize, which may be related to the amount and characteristics of the residues of these crops. The other N-mineralization indices showed values not statistically different between the two sampling dates.

The soil samples from the intensively grazed pasture, collected at different dates, released significantly different amounts of N during the pot experiment and measured as N uptake by turnip. The lower values of N uptake by turnip were recorded from the soil sampled late in spring and in early summer, whereas the greater ones were from the soil sampled in the autumn (Table 3). Inorganic N released by cold and hot KCl and Hyd NH_4 varied also with the sampling date. In contrast, the organic C, Kjeldahl N, $NaHCO_3$-205, and $NaHCO_3$-260 were not statistically different among the soil samples collected on different dates.

The lowest values of N uptake by turnip were recorded in spring and early summer, probably because the easily mineralizable N fraction would have been reduced by the favorable N-mineralization conditions occurring in the field during that period. In the samples taken in winter and late summer, more residues would be accumulated in the soil, because the local conditions (frost in the winter and lack of moisture in the summer) would not favor microbial activity. Thus, the N uptake by turnip from the later soil samples was greater. To a certain extent there was a coincidence between the greater values of Hyd NH_4 and N uptake by turnip, which strengthens the thesis that in spring and early summer the pool of easily mineralizable N is reduced by the activity of microorganisms. Soil inorganic N showed the lowest value in November 2007, probably because of nitrate

Table 2
Nitrogen mineralization indices for the different soil sampling dates in the small grains and maize crops

Sampling date	Organic C (g kg^{-1})	Kjel N (g kg^{-1})	$NaHCO_3$ 205 nm	KCl Nmin (mg N kg^{-1})	HKCl-NH_4 (mg N kg^{-1})	Hyd NH_4 (mg N kg^{-1})	N uptake (mg pot^{-1})
15 Oct. 2008	15.21 a	0.75 a	1.52 a	22.52 a	11.25 a	6.15 a	23.03 b
22 May 2009	15.61 a	0.73 a	1.62 a	18.38 b	10.70 a	4.20 a	31.53 a

Note. Means followed by the same letter in the columns are not statistically different by the Tukey–Kramer HSD test ($\alpha < 0.05$).

Table 3
Nitrogen mineralization indices for the different soil sampling dates in the intensively grazed pasture

Sampling date	Organic C ($g\ kg^{-1}$)	Kjel N ($g\ kg^{-1}$)	$NaHCO_3$ 205 nm	KCl Nmin ($mg\ N\ kg^{-1}$)	HKCl-NH_4 ($mg\ N\ kg^{-1}$)	Hyd NH_4 ($mg\ N\ kg^{-1}$)	N uptake ($mg\ pot^{-1}$)
8 Oct. 2007	10.03 a	0.39 a	1.56 a	15.57 abc	10.10 ab	3.70 a	15.55 ab
8 Nov. 2007	10.85 a	0.45 a	1.56 a	13.63 c	10.40 ab	3.50 a	14.24 ab
17 Dec. 2007	10.91 a	0.42 a	1.39 a	17.88 abc	12.38 ab	1.65 abc	15.58 ab
23 Jan. 2008	9.46 a	0.42 a	1.78 a	18.78 ab	12.20 ab	1.10 bc	14.00 ab
17 Mar. 2008	10.68 a	0.38 a	1.43 a	16.93 abc	13.80 a	2.95 ab	14.97 ab
18 Apr. 2008	9.73 a	0.38 a	1.38 a	15.29 bc	10.70 ab	1.30 bc	12.67 ab
4 June 2008	11.01 a	0.42 a	1.54 a	16.98 abc	11.65 ab	0.55 c	12.87 ab
26 June 2008	10.91 a	0.37 a	1.82 a	18.85 ab	10.40 ab	0.60 c	10.41 b
19 Aug. 2008	10.96 a	0.40 a	1.74 a	17.62 abc	9.00 b	2.60 abc	14.57 ab
1 Oct. 2008	10.43 a	0.42 a	1.57 a	21.24 a	10.78 ab	1.28 bc	19.44 a

Note. Means followed by the same letter in the columns are not statistically different by the Tukey–Kramer HSD test ($\alpha < 0.05$).

leaching by the autumn rains. A high value was found in October 2008, because at this time the heavy rains had not yet arrived and the young plants in the pasture were emerging, still having a limited capacity to absorb nutrients. The lack of significant differences between sampling dates of organic C, Kjeldahl N, $NaHCO_3$-205, and $NaHCO_3$-260 indices may mean that they were less sensitive to changes occurring in the soil over the period of a year. The lack of quantitative meaning in the changes occurring in organic C and Kjeldahl N over the year could be because of the limitations of the analytical methods by which they were determined. The results of Sharifi et al. (2007) showed that $NaHCO_3$-205 and $NaHCO_3$-260 were highly correlated with pool III (the potentially mineralizable N predicted from the fitted curve that did not mineralize during the incubation period). This means that the organic fraction released by 0.01 mol L^{-1} $NaHCO_3$ was provided from the most stable pool of organic matter and may explain the poor sensitivity of the $NaHCO_3$-205 and $NaHCO_3$-260 indices to the changes occurring in the soil over the short term.

In the soil sampled from the olive orchard, the N uptake by turnip was significantly greater in May in comparison to September. The N uptake by turnip was also significantly greater beneath the tree canopy in comparison to the space between rows. No significant differences were found between the two ground-management systems (Table 4). May is a period where there is a great root activity because of the flush of olive growth and the presence of weeds. The root exudates and the residues of weeds and olive trees taken up

Table 4
Nitrogen mineralization indices of soil samples from the rainfed olive orchard

	Organic-C ($g\ kg^{-1}$)	Kjel-N ($g\ kg^{-1}$)	$NaHCO_3$ 205 nm	KCl Nmin (mg N kg^{-1})	HKCl-NH_4 (mg N kg^{-1})	Hyd NH_4 (mg N kg^{-1})	N uptake (mg pot^{-1})
Ground management							
Tillage	13.06 a	0.72 a	1.67 b	30.64 b	13.01 a	4.36 a	37.43 a
PEH	14.44 a	0.78 a	2.02 a	36.65 a	14.00 a	5.03 a	36.51 a
Position in the plot							
Btrees	16.22 a	0.90 a	2.11 a	39.09 a	13.99 a	5.00 a	41.62 a
Brows	11.29 b	0.60 b	1.58 b	28.53 b	13.03 a	4.39 a	32.33 b
Sampling date							
26 Sep. 2008	13.82 a	0.66 b	1.88 a	32.53 a	9.30 b	3.41 b	22.23 b
15 May 2009	13.70 a	0.81 a	1.82 a	34.77 a	16.66 a	5.66 a	51.72 a

Notes. Means followed by the same letter in the columns (within each treatment) are not statistically different by the Tukey–Kramer HSD test ($\alpha < 0.05$). The sources of variation are different ground management systems [tillage and postemergence herbicide (PEH)]; position in the plot [beneath the tree canopy (Btrees) and between rows (Brows)]; and dates of sampling.

in the soil samples would have promoted the net N mineralization in the pot experiment. It is well known that the turnover of fresh biomass is several times faster than that of the other fractions of organic matter (McTaggart and Smith 1993; Shepherd et al. 1996). In the soil samples collected in September, the labile fraction of organic matter would have already been mineralized in the soil, yielding much lower N uptake by turnip. The greater values of N uptake by turnip from the samples collected beneath the tree canopy reflect the progressive accumulation of organic matter in that place. A pool of greater fertility accumulated beneath the tree canopy in the olive orchards of this region was previously reported by Rodrigues, Arrobas, and Bonifácio (2005). The results were attributed to the application of fertilizers beneath the canopy, the increase in the C and N input due to the greater development of the weeds, and the recycling of the nutrients from the falling olive leaves.

In agreement with these findings organic C and Kjeldahl N were significantly greater beneath the tree canopy in comparison to the space between rows. Kjeldahl N was also significantly greater in May, which should reflect the great accumulation of biomass in the soil, in comparison to September. The plot managed with the postemergence herbicide showed slightly greater mean values of organic C and Kjeldahl N, but not statistically different from that of the tillage plot. The $NaHCO_3$-205 and $NaHCO_3$-260 previously related to stable soil organic matter showed results not greatly dissimilar to those of organic C and Kjeldahl N. KCl Nmin was significantly greater beneath the tree canopies in comparison to the space between rows, and greater also in the postemergence herbicide plot in comparison to the tillage plot. No significant differences in KCl Nmin were observed between the two sampling dates. Increased soil fertility found beneath the tree canopy could also be responsible for the greater values of soil inorganic N in that place. HKCl-NH_4 and Hyd NH_4 were significantly greater in May than in September. No significant differences were found between HKCl-NH_4 and Hyd NH_4 in regard to the growth-management systems and when the samples from beneath the tree canopy and between rows were compared. Thus, HKCl-NH_4 and Hyd NH_4 results showed the trend observed in Kjeldahl N and N uptake by turnip.

Conclusions

Nitrogen uptake by turnip in the pot experiment appeared to be a good biological test because it was very sensitive to all the sources of variation included in the experiment. It distinguished well among the different sampling dates of all the agrosystems as well as among the sampling sites in the olive orchard, beneath the tree canopy, and between rows. Kjeldahl N was the chemical test best related to N uptake by turnip when the samples of all the agrosystems were included in the correlation analysis. It would appear to be a suitable N-mineralization index if the nature of the soil samples varies greatly. Within each individual agrosystem the performance of Kjeldahl N was poorer. It did not distinguish among the different dates of sampling in the small grains and maize crops nor among the 10 dates of sampling in the pasture. This analytical procedure was not sensitive to the small variations that would occur in the same soil over a short period of time. Soil inorganic N appeared as a suitable index when the correlation was performed on all the samples of the three agrosystems. It was also a good index of N availability when its performance was analyzed within each agrosystem. Soil inorganic N shows a close correlation with N uptake by turnip and was sensitive to the sampling dates in small grains and maize crops, pasture, and olive orchard. The hot saline KCl extracts appeared to be a fair chemical test when the analysis included the soil samples of all the agrosystems and also when analyzed within each agrosystem. Nothing interesting was recorded regarding the $NaHCO_3$-205 and $NaHCO_3$-260 indices in this experiment.

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