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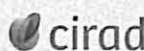
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## Effect of sampling date on chemical indices of the mineralizable nitrogen in soil

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In the 1960s and 1970s there was great emphasis on selecting simple laboratory methods to estimate the potentially available soil N that could be routinely used as a method for the N recommendations systems (Keeney and Bremner, 1966; Stanford and Smith, 1976). Many chemical extractants were investigated (acids, alkali, salts, water, ...) as possible tools for assessing how much N may be mineralized, varying the concentration of the extractant, temperature and time of extractions. The results were usually related to N mineralized in laboratory incubations or with N recovered in field crops. Similar methodologies were also used to predict the N mineralized from organic substrates (manures, sewage sludge, ...). In the following 1980s and 1990s, the published work was still dominated by optimism, with the researchers finding good relationships between chemical indices and plant available N (Gianello and Bremner, 1986). However, after decades of searching for a rapid method to estimate the N mineralization capacity of soil there are still no consistent recommendations. Many conflicting results have been appearing in the literature after a general inability of the following researchers to replicate the positive results however found (Wang et al., 2001). One possible source of error could be the time of soil sampling. It would be expected 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 due to debris deposition and root death. In this work the effect of sampling date on N extracted by several chemical methods was investigated in three different agrosystems: an intensive grazed pasture rich in legs; a rainfed olive orchard, the ground of which was managed by a simple application of glyphosate in April; and an intensive cropping system based on two silage crops per year (small grains from October to May, and maize from May to October).

### Materials and Methods

The chemical extraction methods used in this work were essentially the same as reported by Sharifi et al. (2007) and Soon et al. (2007). Extractable  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  (KClNmin) were determined from 10 g of soil and 40 mL 2M KCl shaken for 1 hour. The suspension was thereafter filtered through a Whatman #42 filter paper. The concentrations of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  in the extracts were determined by UV-vis. spectrophotometry. Hot KCl extractable  $\text{NH}_4\text{-N}$  ( $\text{HKClNH}_4^+$ ) was determined by heating (100 °C) 5 g of soil and 20 mL of 2M KCl for 4 hours in a digestion block. Thereafter the tubes were removed and allowed to cool and the extract filtered through a Whatman #42. Hydrolysable  $\text{NH}_4\text{-N}$  ( $\text{HydNH}_4^+$ ) was obtained by subtracting initial  $\text{NH}_4\text{-N}$  from hot KCl extractable  $\text{NH}_4\text{-N}$ . The ultraviolet absorbance of  $\text{NaHCO}_3$  extracts were prepared by shaking 2.5 g soil with 0.01 mol  $\text{L}^{-1}$   $\text{NaHCO}_3$  for 15 min. The suspension was filtered through a Whatman #42. The ultraviolet absorbance of the extracts was measured at 205 (Abs205) and 260 (Abs260) nm. Kjeldahl N (Kjel-N) was determined after the digestion of 1 g of soil with  $\text{H}_2\text{SO}_4$  and selenium as the catalytic agent in a heated (400°C) aluminium digestion block. After cooling, the suspension was distilled with alkali and the  $\text{NH}_4^+\text{-N}$  in the digest was titrated with HCl in a Kjeltac Auto 1030 Analyzer. Soil samples were provided from an intensive grazed pasture (sampled 10 times throughout the year); a rainfed olive orchard (sampled in October and May); and an intensive cropping system based on small grains and maize (sampled in October and May) grown for silage.

### Results and discussions

The indices based on the UV absorbance at 205 and 260 nm appear very stable over time in all the agrosystems, without significant differences between sampling dates (table 1). Mineral-N released by cold KCl was significantly different between dates of sampling in the winter cereals-maize system and in the grazed pasture. The instability of the inorganic N fraction in soils is well-known and it may decrease quickly due to plant uptake, biological immobilization or by being lost from the

soil. A rapid increase of the mineral N in the soil is also possible when a net N mineralization occurs and there are no favorable conditions for crop uptake or N lost from the soil.  $\text{NH}_4^+$  released by hot KCl was significantly different among sampling dates in the olive orchard and also in the pasture. The  $\text{HydNH}_4^+$  followed the same pattern. In spite of there being no clear reference in the literature as to which organic fraction is preferentially released by saline KCl solutions, it seems that  $\text{HydNH}_4^+$  is readily dependent on soil conditions at the time of sampling. In the pasture the higher values seem to be found in the autumn and early spring. Probably root activity together with microbial activity, which are dependent of environmental variables, were the main factors influencing the  $\text{NH}_4^+$  hydrolyzed by saline KCl solutions. The same seems to be true for the olive orchard, with the root activity in May (olive and weeds) promoting the presence of easily hydrolysable organic compounds.

**Table 1.** N mineralization indices for different soil sampling dates of the agrosystems maize/winter cereals for silage, olive orchard and grazed pasture.

Crop	Date	Abs205	Abs260	Kjel-N	KClNmin	HKClNH <sub>4</sub> <sup>+</sup>	HydNH <sub>4</sub> <sup>+</sup>
				(g kg <sup>-1</sup> )	(mg NH <sub>4</sub> -N kg <sup>-1</sup> )		
Maize	15-Oct-08	1.52 a	0.41 a	0.75 a	22.52 a	11.25 a	6.15 a
	22-May-09	1.62 a	0.51 a	0.73 a	18.38 b	10.70 a	4.20 a
Olive	26-Sep-08	1.76 a	0.41 a	0.70 a	27.74 a	8.67 b	3.60 b
	15-May-08	1.48 a	0.47 a	0.62 a	28.51 a	17.00 a	7.73 a
Pasture	08-Oct-07	1.56 a	0.51 a	0.39 a	15.57 abc	10.10 ab	3.70 a
	08-Nov-07	1.56 a	0.60 a	0.45 a	13.63 c	10.40 ab	3.50 a
	17-Dez-07	1.39 a	0.47 a	0.42 a	17.88 abc	12.38 ab	1.65 abc
	23-Jan-08	1.78 a	0.64 a	0.42 a	18.78 ab	12.20 ab	1.10 bc
	17-Mar-08	1.43 a	0.53 a	0.38 a	16.93 abc	13.80 a	2.95 ab
	18-Abr-08	1.38 a	0.49 a	0.38 a	15.29 bc	10.70 ab	1.30 bc
	04-Jun-08	1.54 a	0.63 a	0.42 a	16.98 abc	11.65 ab	0.55 c
	26-Jun-08	1.82 a	0.62 a	0.37 a	18.85 ab	10.40 ab	0.60 c
	19-Ago-08	1.74 a	0.56 a	0.40 a	17.62 abc	9.00 b	2.60 abc
	01-Oct-08	1.57 a	0.48 a	0.42 a	21.24 a	10.78 ab	1.28 bc

Within each agrosystem means followed by the same letter in the columns are not statistically different by Tukey-Kramer HSD test ( $\alpha < 0.05$ ).

### Conclusion

The results showed that the sampling date may influence the N mineralization indices obtained by chemical extractions, at least when KCl was used as the extracting agent. Thus, it seems that the soil sampling date should be specified in the standard procedures of each method that presents promising results to be adopted in a Lab as a routine procedure.

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