



Relationship between Subsoil Nitrogen Availability and Sugarbeet Processing Quality

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ABSTRACT

The loss of sugar content and processing quality during the harvest period happens frequently in sugarbeet (*Beta vulgaris* L. ssp. *vulgaris*) grown in the Po Valley of Italy. The aim of this study was to assess the consequences on sugar content and quality of excess mineral N in the deep soil explored by the roots. Soil mineral N concentration and other chemical properties to a 3-m depth were correlated with sugar content and processing quality of sugarbeet in 27 sites sampled in 2000–2003. At each site, 12 soil samples with 0.25-m depth increments were collected and analyzed separately. Organic matter as high as 10% and mineral N as much as 100 mg kg⁻¹ frequently were found between 2 and 3 m, corresponding to the maximum depth of the sugarbeet root system. Significant negative relationships were observed between mineral N at the 2.5- to 3-m depth and sugar content ($r = -0.63$) and quality ($r = -0.72$). This study indicates both the utility of soil sampling to the depth reached by the roots, and the need of more complete analyses of mineral N, which should include not only nitrate N, but also ammonium N and organic matter. To reduce the losses of sugar content and quality, the presence of organic layers in the rooting zone should be avoided, or adequately considered in the fertilizer management of the crop.

REDUCTION IN SUGAR CONTENT and processing quality of sugarbeet at harvest is a common problem for farmers of the Po River Valley, Italy. Sugar content refers to the sugar (sucrose) percentage (w/w) in the commercial roots and determines the sugarbeet price. High sugar content is considered a key factor for enhancing the extraction capacity of the factories (Dutton and Huijbregts, 2006). Processing quality is a combination of chemical and physical traits of the roots affecting the sugar extraction rate (Burba and Schiewek, 1993). Being a rather complex characteristic, processing quality is routinely evaluated by quantifying three important nonsugars: K, Na, and α -amino N (Campbell, 2005). Potassium and Na are present in roots in appreciable amounts and interfere with sugar crystallization (McGinnis, 1982). α -amino N is a mix of amino acids with the NH₂ group linked to the carbon chain in the α position, and represents numerous N components of the root including betaine, amino acids (glutamine, glycine, alanine), amides, and nitrate (Burba, 1996). These substances

react or decompose during the processing and are detrimental because they induce formation of ammonia, off-colors, and organic acids in the juices (Dutton and Huijbregts, 2006).

Low sugar content and poor processing quality of the beets are among the causes of the high costs sustained by the Italian sugar industry. The reduction in sugar content and quality during harvest, common in Mediterranean countries, occur also when the soil N exceeds the crop uptake, suggesting that excess N might contribute to these losses in fields where otherwise adequate fertility recommendations have been applied (Marchetti et al., 2002). Nitrogen in the soil is present in different forms. Organic N is a mixture of compounds at different stages of oxidation and molecular weight. Mineral N is the sum of NH₄-N and NO₃-N, and represents the bulk of N readily available for crops (Cariolle and Duvall, 2006). Ammonium N displays a very low mobility in the soil and can be absorbed by clay particles becoming unavailable for plants (Marschner, 2003). In the upper part of the soil profile, the concentration of NO₃-N is usually much greater than NH₄-N and represents the major N source for sugarbeet (Draycott, 1972). Nitrate N moves easily into the soil and can be leached by percolating water. For deep-rooted crops, mineral N accumulation in the deeper soil layers is a potentially important source of N (Thorup-Kristensen, 2006). Excess N is more harmful to sugarbeet than to most other crops (Biancardi et al., 1998) because it stimulates vegetative growth, severely reducing both sugar content and quality (Cariolle and Duvall, 2006). Sugarbeet fibrous roots can extend to a depth of 2.8 to 3.0 m in good soil conditions (Märländer and Windt, 1996; Biancardi et al., 1998), and a study using labeled ¹⁵N showed that the crop can accumulate N from depths > 1.8 m (Peterson et al., 1979). Other experiments utilizing ¹⁵N demonstrated that sugarbeet absorbed between 50 and 80% of applied N from depths down to 1.2 m (Haunold, 1983; Broeshart, 1983). Deep fibrous roots, despite being a small fraction of entire sugarbeet root system (Vamerli et al., 2003), are critical in depleting deep mineral N,

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as observed in vegetables by Kristensen and Thorup-Kristensen (2004). In the Po Valley, fibrous roots of sugarbeet reach the deeper soil horizons toward the end of July at the beginning of the harvest (Marchetti et al., 2002), when excess N has detrimental effects on sugar content and quality (Blumenthal, 2001). During this period, sugarbeet should become N-deficient to achieve the best levels of quality (Winter, 1998; Blumenthal, 2001).

Growers need to provide enough N to ensure optimal sugar yield and quality at the lowest environmental and production costs, while minimizing overfertilization effects (Lobell, 2007). Correct evaluation of this N balance often is difficult partly because the assessment is based on the sole analysis of NO₃-N content (Bilbao et al., 2004). The major challenge is to estimate the mineralization rate of organic matter, which can supply as much as 60 kg ha⁻¹ yr⁻¹ (Draycott and Christenson, 2003; Jaggard et al., 2009). Other methods to quantify N fertilizer requirements, such as plant tissues analyses, chlorophyll content, or remote sensing, have not always been reliable (Draycott and Christenson, 2003).

Over the last 30 yr, N fertilization of sugarbeet was reduced appreciably because of the increasing costs for fertilizers and the requirements of the factories for higher processing quality (Märländer et al., 2003). Nevertheless, overfertilization is still frequent, especially in clay and organic soils or where the residual mineral N was not properly quantified (Martin-Olmedo et al., 1999). Biancardi et al. (2008) observed typical symptoms of excess N in sugarbeet variety trials even without the application of any fertilizer. These authors hypothesized that the excess N was due to mineral N accumulation beneath

the sampled layer, which is the plowed soil (0–0.5 m). The need for more representative soil analyses slowly is becoming accepted (Thorup-Kristensen et al., 2009). Indeed, the recommendation for the sampling depth increased over time from 1.5 to 1.8 m (Reuss and Rao, 1971; Blumenthal, 2001), but often, in the normal practice, only the plowed layer is sampled (Martin-Olmedo et al., 1999). The aim of the present study was to test the hypothesis that unforeseen amounts of mineral N in the soil below the routinely sampled layer could explain the decrease of sugar content and processing quality, frequently observed during harvest of sugarbeet.

MATERIALS AND METHODS

Study Area

The research was conducted at 27 sites in the eastern Po River alluvial plain in Italy (Table 1). The sites were selected out of variety trials performed yearly by the Italian sugarbeet growers associations and the sugar industry. The fields were fertilized before sowing based on analysis in the 0- to 0.5-m soil layer taken in October following local recommendations (BETA, 2009). The climate in the Po River Valley ranges from temperate suboceanic in the internal areas, to warm temperate-oceanic and partly sub-Mediterranean in the coastal area (Costantini et al., 2004). Annual average temperature in the area ranges from 11 to 13°C, and annual average precipitation varies from 690 to 1200 mm concentrated mainly in May and October, whereas the driest months are July and August. The soils of the lower Po Valley derive from quaternary alluvial and glaciofluvial deposits with mean slopes around 1%, and are frequently characterized by organic matter or peat layers with variable depth and thickness. The texture of soils was mainly silt-loam, but varied greatly among the experimental sites. Mean value of soil pH was 8.1, ranging from 5.5 to 8.9 over all the sampled layers. The soil pH of some layers was below 7.0 only when the organic matter was high.

Soil Sampling

Soil samples were taken in May and June in the center of each selected variety trial to a depth of 3.0 m at 0.25-m increments. These months were chosen to facilitate deep soil sampling by avoiding effects of surface water table and/or impenetrable layers following drought periods. In this period, the crop is at the stage of 8 to 15 pairs of true leaves. The samples were taken by means of an Edelmann 36-mm diameter manual auger (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). In some cases, the presence of ground water and hard or sandy layers caused difficulties in extracting the deepest cores. Additionally, several trials were not harvested due to disease, drought, or low uniformity. Consequently, a complete set of soil and sugarbeet data corresponding to 27 sites was used (Table 1).

Soil Analyses

Soil samples were frozen and stored at –20°C until analysis. Organic matter was measured on air-dried samples by means of the potassium dichromate titration method of Walkley and Black (Page et al., 1982). Mineral N was extracted from the soil using a 2 M potassium chloride solution (1:5 soil/solution ratio). Nitrate N was reduced to NO₂-N by passing the

Table 1. Year of sampling, geographic coordinates, and altitude of 27 sites in the Po Valley of Italy, where the relationship between deep mineral N concentration and sugarbeet processing quality were studied.

Location	Year	Latitude	Longitude	Altitude
				m
1 Ceregnano	2000	45°02' N	11°51' E	1
2 Filetto	2000	44°20' N	12°04' E	8
3 Lavezzola	2000	44°33' N	11°52' E	2
4 Mirabello field I	2000	44°50' N	11°27' E	8
5 Ostellato	2000	44°44' N	11°56' E	3
6 Rovigo field 40	2000	45°04' N	11°45' E	1
7 Fusignano	2001	44°27' N	11°57' E	6
8 Imola	2001	44°22' N	11°41' E	48
9 Montagnana	2001	45°14' N	11°28' E	11
10 Rovigo field 53	2001	45°04' N	11°45' E	1
11 Trecenta	2001	45°01' N	11°27' E	8
12 Arquà Polesine	2002	45°00' N	11°44' E	2
13 Borgofranco sul Po	2002	45°02' N	11°12' E	11
14 Conselice	2002	44°31' N	11°50' E	2
15 Malborghetto	2002	44°52' N	11°44' E	2
16 Rovigo field 10	2002	45°04' N	11°45' E	1
17 Rovigo field 18	2002	45°04' N	11°45' E	1
18 Rovigo field 63	2002	45°04' N	11°45' E	1
19 San Martino di Venezze	2002	45°07' N	11°52' E	2
20 Salara	2002	45°00' N	11°25' E	6
21 San Marino	2002	44°36' N	11°25' E	13
22 San Pietro in Casale	2002	44°42' N	11°23' E	13
23 Tribano	2002	45°12' N	11°49' E	4
24 Cà Bosco field I	2003	44°28' N	12°09' E	3
25 Cà Bosco field 6	2003	44°28' N	12°09' E	3
26 Rovigo field 20	2003	45°04' N	11°45' E	1
27 Rovigo field 60	2003	45°04' N	11°45' E	1

potassium chloride solution through a cadmium column. Following Keeney and Nelson (1982), $\text{NH}_4\text{-N}$ was determined colorimetrically with sulphanylde and dichloroisocyanuric acid, respectively, using an Autoanalyzer 3 (Bran & Luebbe GmbH, Norderstedt, Germany). Soil pH was measured in 1:2.5 soil/water solution with a glass electrode. The mean amounts of mineral N ha^{-1} in each 0.25-m layer were calculated using a soil bulk density of 1.35 Mg m^{-3} , which is the average value in the sampled area.

Sugarbeet Analyses

In each field trial, 7200 topped sugarbeet were collected and analyzed. After washing the roots, from this large sample, about 1 kg of micronized tissues (brei) was obtained with a special sawing machine (AMA-KWS, AMA Werk GmbH, Alfeld, Germany). About 70 g of representative homogenized brei samples were immediately frozen at -40°C . Sugar content and the main nonsugars were analyzed after cold digestion of the brei in lead acetate 0.75% (w/w) solution (Schneider, 1979) using an automated brei-mixer (Venema Automation b.v., Groningen, the Netherlands). Sugar content was determined using a Thorn-Bendix 243 polarimeter (Bendix Corp., Nottingham, UK). Potassium and Na concentrations were measured with a flame photometer (Model IL 754, Instrumentation Laboratory S.p.A., Milan, Italy). The α -amino N was quantified by colorimetric analysis (PM2K; Carl Zeiss GmbH, Oberkochen, Germany) following the procedure proposed by Kubadinow and Wieninger (1972). Processing quality (purity) was calculated according to Wieninger and Kubadinow (1971). In this case, purity represents the percentage of sugar from the roots extractable by the factory:

$$\text{AC} = \frac{\text{Na} + \text{K}}{\alpha\text{-amino N}}$$

$$\text{if AC} < 1.8 \text{ then } \text{MZ} = 0.86 \times 0.3423 \times \alpha\text{-amino N}$$

$$\text{if AC} \geq 1.8 \text{ then } \text{MZ} = 1.02 \times 0.3423 \times (\text{K} + \text{Na})$$

$$^\circ\text{S extr.} = \frac{100 - \text{MZ}}{100} \times ^\circ\text{S}$$

$$\text{Z tot.} = \frac{\text{root yield}}{100} \times ^\circ\text{S}$$

$$\text{Z extr.} = \frac{\text{root yield}}{100} \times ^\circ\text{S extr.}$$

$$\text{Purity (\%)} = \frac{\text{Z extr.}}{\text{Z tot.}} \times 100$$

where AC is the alkalinity coefficient; Na, K, and α -amino N are expressed as millimoles 100 g^{-1} sugar; MZ is molasses sugar; $^\circ\text{S}$ is sugar percentage (w/w) in the roots; $^\circ\text{S extr.}$ is extractable sugar percentage; Z tot. is gross sugar yield (tonnage of roots per hectare $\times ^\circ\text{S} \times 100^{-1}$); and Z extr. is extractable sugar yield (t ha^{-1}).

Statistical Analyses

One-way ANOVA and regression analyses were performed in SAS 9.1 (SAS Institute, 2002). Analysis of variance was used to test if nutrient concentrations were different depending on the soil layer. Data were tested for homogeneity of variance before statistical analysis (Levene, 1960) and were transformed (either square root or logarithm) when the normal distribution assumption was violated. Significant ($P < 0.05$) treatment effects were explored further via a posteriori treatment comparison with the least-squares means test and the Bonferroni adjustment for multiple comparisons. Linear regression analyses were performed using the PROC REG procedure in SAS, with sugar content or quality as the dependent variables and soil parameters ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and mineral N contents) as independent variables. The data of 27 soil profiles, collected in different locations and years (Table 1), and the production traits of the corresponding trials were used in the regressions. To obtain more representative values of mineral N and organic matter, regression analyses were performed using average values of 0.5-m soil layers. Regressions parameters obtained are shown in Table 2.

Table 2. Intercepts (a), slopes (b), and coefficients of determination (R^2) for linear regressions of main nonsugars and purity and the mineral N and ammonium N ($\text{NH}_4\text{-N}$) content in the soils of 27 sites. Significant relationships ($P < 0.05$) are shown in bold.

Soil depth	Sugar content			Sodium			α -amino N			Purity		
	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2
m												
$\text{NH}_4\text{-N}$												
0.00–0.50	14.227	0.227	0.005	1.879	0.273	0.009	2.684	0.084	0.030	85.440	-0.168	0.002
0.50–1.00	14.240	0.338	0.004	2.057	0.002	0.007	2.480	0.275	0.032	84.482	1.577	0.007
1.00–1.50	14.770	-0.674	0.036	2.008	0.092	0.006	2.200	0.835	0.059	85.761	-0.823	0.104
1.50–2.00	14.716	-0.527	0.116	1.948	0.194	0.102	1.866	0.379	0.108	86.486	-2.073	0.217
2.00–2.50	14.995	-0.069	0.344	1.564	0.059	0.350	2.125	0.061	0.372	87.769	-0.295	0.425*
2.50–3.00	15.065	-0.066	0.407*	1.425	0.065	0.519**	1.981	0.067	0.449*	88.230	-0.297	0.623**
Mineral N												
0.00–0.50	14.957	-0.122	0.019	1.642	0.095	0.015	1.606	0.235	0.077	88.761	-0.787	0.060
0.50–1.00	16.211	-0.409	0.142	1.415	1.147	0.024	0.590	0.468	0.199	92.235	-1.581	0.159
1.00–1.50	15.154	-0.201	0.042	1.905	0.041	0.102	1.525	0.304	0.102	87.953	-0.720	0.102
1.50–2.00	13.987	-0.150	0.215	1.820	0.263	0.160	1.800	0.058	0.092	82.290	-0.050	0.254
2.00–2.50	15.288	-0.070	0.355	1.391	0.054	0.353	1.843	0.064	0.404	88.763	-0.279	0.536
2.50–3.00	15.174	-0.060	0.398*	1.395	0.053	0.406*	1.848	0.063	0.465*	88.458	-0.250	0.518**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

RESULTS AND DISCUSSION

The mineral N content in the plowed layer across all sites was 13.1 mg kg⁻¹ of dry soil (Fig. 1a). Mineral N content was different depending on the soil layer ($P < 0.0001$). It significantly decreased up to 2 m depth and afterward rapidly increased in the layers deeper than 2.0 m (Fig. 1a). The mineral N was mainly composed (about 94%) of NO₃-N up to a depth of 1 m (Fig. 1b). Below 1 m, NO₃-N decreased progressively. Ammonium N content was about 22 times less than NO₃-N in the upper 1 m, representing a small fraction of mineral N until the 2-m depth (Fig. 1c). About one third of the sites had a high concentration of NH₄-N in the 2- to 3-m layers relative to the upper soil. Owing to the low NH₄-N mobility, these values were associated with the high contents of organic matter or peat (Fig. 1d). Similar effects of organic matter on NH₄-N concentration were observed by Evangelou et al. (1986).

The sum of the mineral N amounts present in the entire rooting profile (478 kg ha⁻¹, Fig. 1a) indicated that the mineral N was far above the crop N requirement, which is

approximately 200 kg ha⁻¹ before sowing and 100 kg ha⁻¹ in the middle of the growing season (Draycott, 1993). In only one case over the 27 sites, the quantity of mineral N in the rooting zone was below the limit of 100 kg ha⁻¹. Thus, the majority of the sampled soils could be considered as having an excess of mineral N. It should be noted that fields were sampled in May and June, at the beginning of the period of more intense mineralization of organic matter. Since soil temperature increases during the summer, it is expected that N mineralization and the amount of mineral N for beets increased in the second half of the growing season (Bilbao et al., 2004; Tsialtas and Maslaris, 2005).

Organic matter in the deep profiles (2–3 m) of these soils was strongly correlated with mineral N and NH₄-N ($r = +0.86$, $P < 0.001$; and $r = +0.91$, $P < 0.001$, respectively) and may be regarded as the primary source of mineral N. No relationships were found between organic matter and NO₃-N in the entire soil profiles. Based on the mineral N concentration found in October as part of the standard fertilization

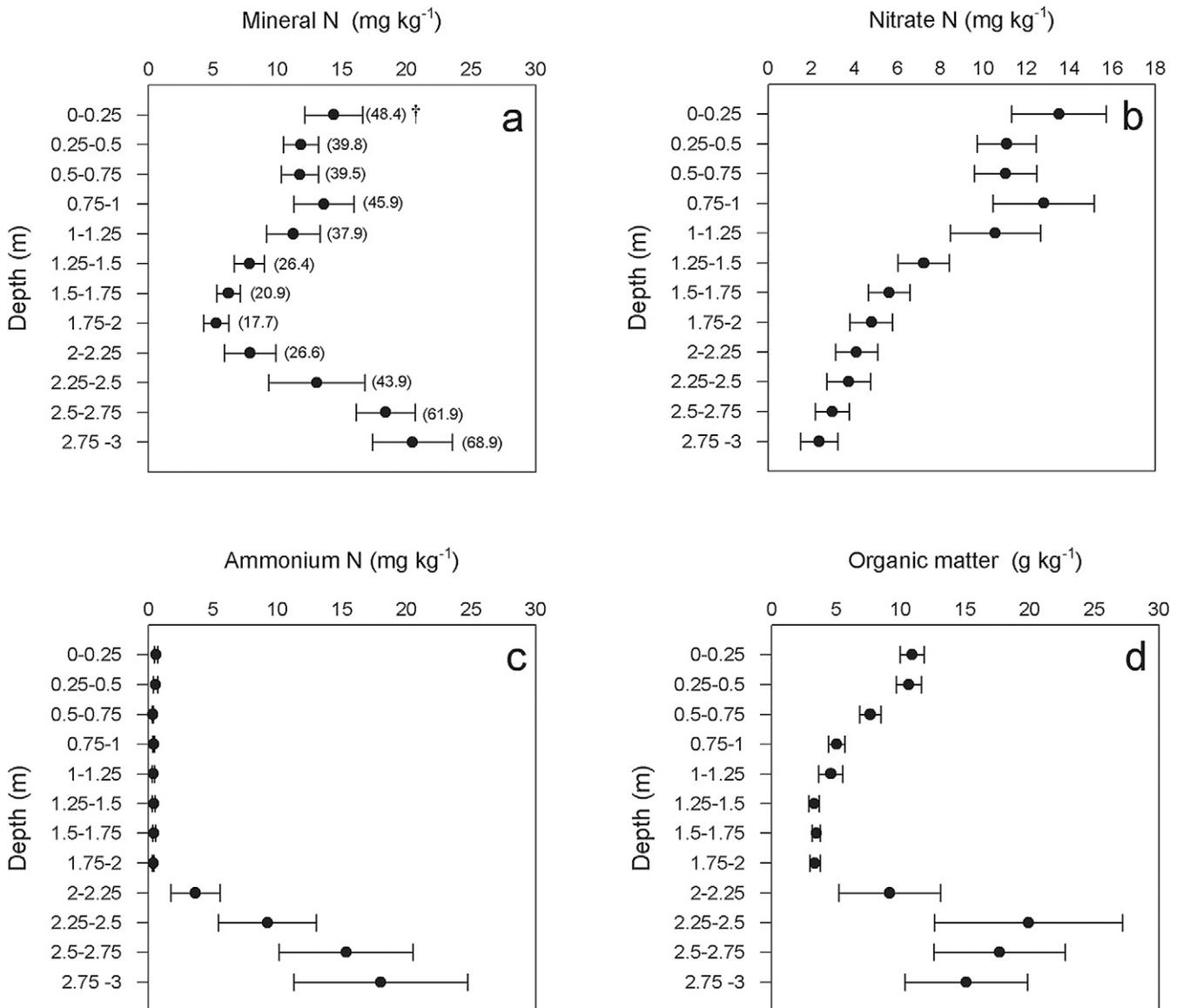


Fig. 1. Total mineral N, NO₃-N, NH₄-N, and organic matter content of the soil profiles averaged across 27 sites in the Po Valley of Italy. Bars represent the standard error of the mean values. † Amounts of mineral N in kg ha⁻¹ are shown in parentheses.

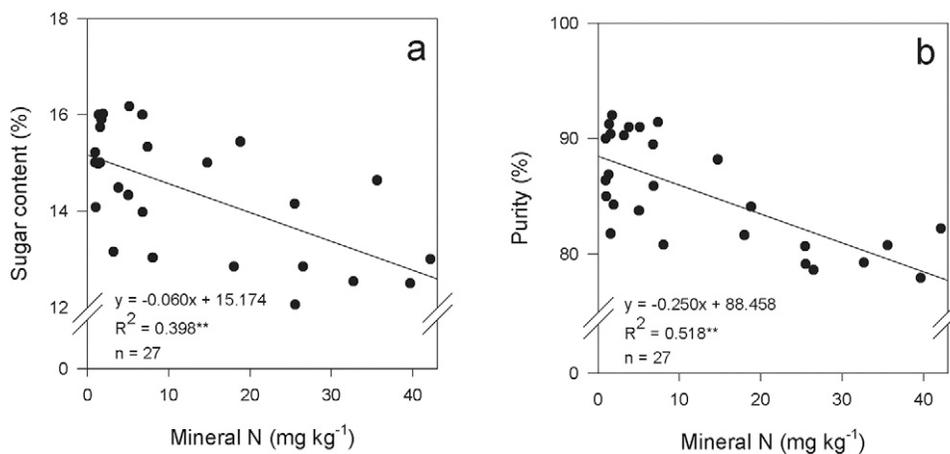


Fig. 2. Relationships of sugar beet sugar content and purity (Wieninger and Kubadinow, 1971) with soil mineral N at the 2.5- to 3.0-m depth in the Po Valley of Italy during 2000–2003. The data points represent the average value of each plot.

practice (calculated on samples taken in the 0–0.5 m) all the 27 sites would require addition of fertilizer to residual N. When considering soil samples from the upper 1.5 m layer, N fertilizers would be necessary in only 15 of the 27 sites. Considering the entire rooting zone (0–3 m) further reduced the number of fields that would benefit from additional N fertilizers to only one (Biancardi et al., 2008). Therefore, the threshold between applying or not applying fertilizers, as well as the amount of N to apply, would depend mainly on the sampling depth.

Linear regression analyses showed significant relationships between root traits (sugar content, Na, α -amino N, and purity) and the $\text{NH}_4\text{-N}$ and mineral N concentration, but only in the deepest layers (Table 2; Fig. 2). Sodium and α -amino N increased, whereas sugar content and purity diminished with rising mineral N concentration. Nitrate N did not affect the processing quality parameters. Mineral N influenced root K concentration less than Na and α -amino N (Tsiatas and Maslaris, 2005). Given that the $\text{NH}_4\text{-N}$ mobility in the soil is negligible, these significant correlations only at the greatest depth support previous observations, indicating that sugarbeet roots can absorb nutrients at depths up to 3 m and that, in the period close to harvest, N is acquired through the deep root system. When mineral N exceeds 20 mg kg^{-1} at the 2.5- to 3-m depth, the level of sugar content and purity tended to be <14% and 85%, respectively, which are acceptable thresholds for farmers and processors in the area studied (Casarini et al., 1999). Owing to the influence of deep mineral N on the commercial value of sugarbeet, the guidelines currently given for the determination of N fertility should be modified by including the deep soil explored by the fibrous roots. Of course, adaptation of the sampling depth to the local soil conditions and crop requirements is necessary in each cultivation area. The deep layers can be easily accessed using powered augers (Cariolle and Duvall, 2006).

CONCLUSIONS

The analyses of soil samples taken from 27 sites to the 3-m depth allowed an assessment of the vertical N distribution in soils of the eastern Po River Valley. Relatively high organic matter content was frequently detected between the 2- and 3-m depth and was associated with elevated mineral N

concentration, $\text{NH}_4\text{-N}$ in particular, at these depths. These unforeseen amounts of mineral N were correlated with the drop of sugar content and processing quality. For an accurate evaluation of the residual N, which determines not only the need but also the amounts of N fertilizers, the soil sampling depth must be adequately increased according to the local pedoclimatic situations. Additionally, the analysis of $\text{NO}_3\text{-N}$ should be integrated with those of $\text{NH}_4\text{-N}$ and organic matter. Because of the unpredictable rate of mineral N release during the growing season, the presence of layers with a high content of organic matter up to the depth reached by the roots should be taken into account in the N fertilizers management.

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