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Organic Wastes Use in Horticulture: Influences on Nutrient Supply and Apple Tree Growth

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Authors' contributions

This work was carried out in collaboration between all authors. Author MK designed the study. Author s EL and SP carried out the field and laboratory work. SP managed the literature searches and the statistical analyses. SP and DN wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The application of fertilizers derived from agroindustrial by-products, represents an interesting opportunity in organic farming. The composition of such inputs includes complex molecules, which strongly influence their effectiveness in providing the optimal nutrient availability and improving crop performances. The aim of the present study was to determine the nutrient release rate of different organic fertilizers and amendments and their effects on shoot growth, fruit production, leaf nutritional status, root biomass and morphology. In a two-years pot trial, two organic fertilizers produced with dried fungal biomass (DFB) and vinasse of sugar beet pulp (VN) and two amendments obtained from fermented animal sewage (SE) and composted olive husks plus grapevine waste (OG), were applied on apple rooted cuttings. One set of plant were not fertilised and acted as Control. The application of DFB, VN and SE increased nitrogen concentration in the soil and in the leaves, supported higher fruit number and enhanced plant growth above and below ground compared to OG and Control. The effect on root growth was positively correlated with nitrogen mineralization rate. For OG treatment, soil electric conductivity negatively influenced root branching frequency, indicating a potential risk of stress due to salinity excess.

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1. INTRODUCTION

The practice of organic farming, as conservative agricultural method, has increased exponentially worldwide [1]. The agricultural use of organic residues offers an attractive option for their safe disposal and a valuable source of organic amendments and nutrients. Sewage sludge, wine and oil mill waste, and vinasse are among the by-product potentially suitable for fertilizers production. Yet despite the apparent benefits, the widespread adoption of organic inputs has been limited, due in part to a lack of unbiased scientific research into the agricultural effectiveness of these products [2]. A lack of knowledge over the mineralization dynamics of most of organic fertilisers and amendments in soil is an obstacle to their correct application and may limit their efficacy. Most of the fertilizers and amendments, which can be used in organic farming, contain nutrients within organic molecular structures; these nutrients are available to plants only after they undergo a complex mineralization process [2]. The apparent deficiency of an adequate supply of plant-available nutrients from organic fertilizers, resulting from a slow rate of mineralization, often makes lower crop yields in fields treated with organic fertilizers compared to those treated with chemical inputs [3]. Furthermore, several studies [4,5,6] have reported, for organic inputs, features considered as limiting factors for their horticultural use, such as the presence of hazardous components (i.e. heavy metals), phytotoxic compounds [7,8], or an excess in salts or nutrients that originate media with limiting electrical conductivity EC [9].

Recent studies tested the efficiency of some organic by-products as an alternative to traditional peat growth media [10]; however clear relationships to plant yield [11] are lacking for the use of the same products as fertilizer or amendment. Literature about the consequences of fungal biomass application to crop is scarce nowadays. Results showed that sewage sludge might increase plant available nitrogen (N) and phosphorous (P) [12,13] and provide large amounts of organic matter [14]. A growing interest is also in the exploitation of the residues generated by the wine industry and oil mill [15,16], since interesting improvement on plant performances were demonstrated after their application [17]. As a counterpart, elevated EC was found in soil as a limiting factor for salt sensitive crops, after the application of compost derived from such by-products [18]. The use of beet vinasse has showed variable success [19]. The high levels of N (30 g kg^{-1}), K (30 g kg^{-1}) and organic matter (350 g kg^{-1}) of the concentrated vinasse can be beneficial factors in recycling this waste for agricultural purposes, but the direct application of vinasse might generate short comings due to its high salinity and P content [20]. During a study conducted on ryegrass *Lolium multiflorum* the application of vinasse inhibited germination, root elongation and early seedling emergence when performed at sowing time [21].

The present study was designed to compare two different amendments and two fertilizers derived from agroindustrial by-products and applicable in organic farming. The research aimed to determine their nutrient release dynamics and their effect on soil chemical fertility and plant performances trying to reveal any restraining effects that could represent a limitation on their use.

2. MATERIALS AND METHODS

2.1 Plant Material and Organic Inputs

Rooted cuttings of apple clonal M9 rootstock with diameter of 6-8 mm were cut at 25 cm, leaving two bud and planted in 27-L pots filled with sandy-silt soil. Over the course of two years, these plants were supplied with four different nutrient inputs that are permitted for use in organic farming (Table 1). Two of these have N concentrations >3%, and they are therefore considered as fertilizers: a granulated product obtained from dried fungal biomass (DFB) and the final by-products obtained from pressing sugar beet pulp, known as vinasse (VN). The other two products have N concentrations <3%, and they are therefore considered as amendments: the sewage as derived from anaerobic fermentation of animal waste to produce biogas (SE), and the dried pelleted compost of olive husks and grapevine waste (OG). Fertilizers and amendments N content was measured using the Dumas method [22] and inputs were applied every fortnight, for a total of 8 g N/plant/year from late April to late May of each year. One additional set of plants was not supplied with any fertilizer during the whole trial, and functioned as Control. A randomized block design was used to compare the five treatments in four blocks, with four plants per treatment per block: 16 plants per treatment in total. For the entire study period, the potted apple trees were positioned within the trial orchards of the Laimburg Research Centre (Ora, Italy), and they were daily drip irrigated to field capacity. All measurements on plant and on soil parameters were performed in the second year.

2.2 Estimation of Nutrient Release Rate

The N release rate was estimated by applying the equivalent quantity of the tested organic fertilizers and amendments to 25 g sandy silt soil samples kept in an incubator under standard laboratory conditions (soil water content: 70% of the water holding capacity and temperature: 16°C). The incubator was periodically opened for aeration and the soil moisture was maintained by addition of deionized water. To determine the total N release rate, the soil concentrations of NO₃⁻ and NH₄⁺ were measured at 14 and 60 days after the input application [23] in 4 samples per treatment pooled together. NO₃⁻ and NH₄⁺ were extracted in a solution of 0.0125 M of CaCl₂ and determined colorimetrically by continuous flow analysis (CFA) according to the official VDLUFA guidelines [24].

2.3 Soil Mineral Fertility and Plant Nutritional Status

The soil concentrations of total N were measured also under field conditions each month, from June to October, on soil derived from four sub-samples per treatment mixed together. N determination was performed according to the Dumas method [25] and replicated four times. On October 21 of the second year the soil concentrations of all of the main macro and micronutrients were measured: P₂O₅, K₂O, Mg, B, Mn, Cu and Zn. P₂O₅ and K₂O were extracted in 100 ml of calcium and ammonium lactate solution, pH 4,2 [26]. P was determined colorimetrically and K by a flame photometer. Mg, B, Mn, Cu, Zn were extracted in 100 ml Calcium chloride and diethylenetriaminepentaacetic acid (DTPA) solution and determined by ICP-OES (inductively coupled plasma optical emission spectrometry) [27]. The total soluble salts were also determined on July 31 and October 21 by diluting 20g of soil in 200ml of deionized water according to the official VDLUFA Methodenbuch, I A 10.1.1 guidelines (1991). Soil EC was indirectly estimated from soil soluble salts by considering a conversion factor of 640 (i.e. 640 ppm = 1 dS/m) [28]. The leaf nutrient concentrations were measured on June 9 (fruit set) and September 2 (pre-harvest) on the youngest mature fully

expanded leaves. N concentration was measured according to Dumas method [29]. P, K, Ca, Mg, B, Fe, Mn, Cu and Zn concentration was determined by microwave assisted acid digestion method EPA 3052 [30] and measured by ICP-OES. On four plants per treatment, five leaves were harvested and pooled to constitute a sample used to conduct the nutrients analysis.

2.4 Vegetative Growth and Crop Yield

Shoot and trunk diameter growth, flowering and fruit number were measured in the second year, for each of the 16 plants per treatment. The shoot lengths were recorded monthly from June 19 to October 9 on 5 shoots per plant, and the trunk diameter was measured on June 25 and October 17 at 10 cm from the ground. For single growth intervals shoot elongation was expressed as the relative growth rate (RGR) respect to the previous values. The trunk caliper growth was expressed as percentages of the initial diameter recorded in June. The number of flower clusters (with 5 flowers per cluster on the average) per plant were counted in April and the number of fruits were counted on May 23 and after the June drop on July 15.

2.5 Root Biomass and Morphology

In November of the second year, 160 days after the last input application, a single plant per block per treatment (four trees per variant) was extracted from the pot and the roots were washed free of soil. Total root dry weight was measured. For root morphology measurements, only living, intact and healthy roots were considered in the analysis, whereas all the damaged, shrunken or partially decomposed roots being discarded. The whole root system was separated into five root orders according to Fitter [31], from 1st to 5th, using the root diameter as the discriminant when the original architecture was compromised, and thus not recognizable. All of the 1st and 2nd order roots were combined for the analysis and generally considered as “fibrous” roots (root diameter 0.26 ± 0.02 mm), while roots from the 3rd to 5th orders were kept separated. Total root dry weight was measured for each plant and the relative contribution of the fibrous fraction to the total biomass was expressed as a percentage by separating the 1st and 2nd order root biomass from the rest. The length of the longest root for each plant was measured after the root system has been washed free from soil and positioned on a horizontal surface. The diameter, length and dry weight of a sub-sample of 30 roots per order were measured for each plant. For 5th order class 30 roots were not always available: all available roots were used for analysis in such cases. When the number of available roots exceeded the 30 units, the subsample was composed by randomly selected roots. The root length and diameter were measured by processing images of the root samples through the DigiRoot™ software (Digital processing solutions, CO, USA) for image analysis. The root lengths and dry weights were used to estimate the specific root length (SRL). The lateral branching rate was measured on the same root samples, for roots from 3rd to 5th order, as the number of laterals produced per unit length.

2.6 Statistics

The data were analyzed using ANOVA and the means were separated according to the Tukey's honestly significant difference (HSD) test $p = 0.05$. For leaf nitrogen concentration and shoot growth a two-way ANOVA was performed in order to describe the effect of the nutrient supplied (Treatment), the date of sampling (Date) and their cross interaction Treatment x Date. Statistical analysis were performed by using JMP 9 (SAS Institute Inc., Cary, NC).

3. RESULTS AND DISCUSSION

3.1 Nutrient Release and Soil Mineral Fertility

Under laboratory conditions, VN and SE showed the highest rates of mineralization, with >50% of the N mineralized after 14 days (52.2% and 50.3% respectively). At the same sampling time DFB and OG had 17.7% and 11.5% mineralized N, respectively. After 60 days from application, the low release rate had persisted for OG but had recovered for DFB (Table 1).

Table 1. Principal characteristics of the tested organic fertilizers and amendments: description, total N content, quantity supplied to each plant per year, and % of mineralized N in the laboratory condition after 14 and 60 days

Fertilizer/ amendment (form)	Description	Total N content (%)	Input (g/pot/year)	Mineralized N (%)	
				14 d	60 d
DFB(pellet)	Fertilizer: Dry fungal biomass	6.6	121.2	17.7	38.5
VN(liquid)	Fertilizer: Vinasse from sugar beet pulp.	3.2	248.4	52.2	54.6
SE(liquid)	Amendment: Fermented Sewage	0.34	2352.9	50.3	56.9
OG(pellet)	Amendment: Composted olive and grape waste	0.7	1159.4	11.5	14.2

On field conditions, the highest N mineralization took place in June when VN, DFB and SE showed N content values significantly superior to Control (Table 2). The differences attenuated during the season and were not significant by October (Table 2).

Table 2. Soil EC (dS/m) as measured during the second year on July 31 and October 21 and N content (mg/kg) in field condition over the season. Within each date of sampling values followed by the same letter do not differ significantly from one another, according to Tukey's HSD tests (p = 0.05).

Fertilizer/ Amendment	Soluble salts (dS/m)		N content (mg/Kg)			
	July	October	June	July	August	October
Control	0.92	0.95	0.75±0.2 ^c	1.0±0.1 ^b	0.7±0.2 ^b	1± 0.1 ^a
DFB	1.05	1.33	43.5±8.7 ^{ab}	7.2±1.4 ^a	3.0±0.8 ^a	1.7±0.4 ^a
VN	1.30	1.30	61.7±6.9 ^a	3.0±0.2 ^b	1.0±0.2 ^b	1±0.1 ^a
SE	1.33	1.31	34.7±5.8 ^b	2.6±0.2 ^b	1.7±0.2 ^{ab}	1.5±0.2 ^a
OG	2.60	1.47	2.5 ±0.6 ^c	1.0±0.1 ^b	0.7±0.2 ^b	1±0.1 ^a
SEM ^a	±0.30	±0.09				

^{a)}SEM: standard error of the means.

The application of OG resulted in the highest soil soluble salts concentration both in July and in October (Table 2). For control and plants fertilized with DFB, VN and SE the estimated soil EC never overcame the thresholds indicated for any salinity stress for apple [32,33]. Only for OG in July the EC value reached a level indicated as potential cause of yield loss for apple, being 2.3 dS/m (Table 2).

The OG treatment also induced the highest soil content of P2O5 (Table 3).

Table 3. Soil concentrations of P2O5 and K2O (mg/100g), Mg, B, Mn, Cu and Zn (mg/Kg), as measured during the second year on October 21

Fertilizer/ Amendment	P ₂ O ₅	K ₂ O (mg/100 g)	Mg	B	Mn	Cu	Zn (mg/kg)
Control	14	16	20	0.17	23	16	7
DFB	20	17	19	0.22	28	15	8
VN	13	38	19	0.24	31	13	7
SE	35	43	24	0.31	30	16	11
OG	53	45	21	0.69	22	16	23
SEM ^a	±7.60	±6.35	±0.93	±0.09	±1.83	±0.58	±3.04

^aSEM: standard error of the means.

3.2 Plant Nutritional Status, Growth and Yield

DFB, VN and SE application increased the leaf N concentration through all the season. Only for plants treated with OG, N concentration did not differ from control, especially during the fruit set, in June (Table 4).

Table 4. Leaf N concentrations (on dry weight) measured during fruit setting (June 9) and before harvest (September 2) of the second year ±standard error (SE) According to the two-way ANOVA the nutrient supplied (Treatment) and the date of sampling (Date) influenced the leaf N concentrations as follows: Treatment (p <0.0001), Date (p =0.4501), Treatment × Date (p <0.0001). Within each treatment differences due to sampling date were significant (*) or highly significant () in all cases. Within each date of sampling values followed by the same letter do not differ significantly from one another, according to Tukey's HSD tests (p = 0.05).**

Treatment	Leaf N concentrations (Percent by Dry Weight) ^a		
	9 June	2 September	
Control	1.20±0.04 ^b	1.72±0.16 ^b	*
DFB	2.43±0.04 ^a	2.07±0.04 ^a	**
VN	2.51±0.04 ^a	2.08±0.03 ^a	**
SE	2.31±0.07 ^a	1.94±0.08 ^{ab}	**
OG	1.26±0.09 ^b	1.72±0.01 ^b	**

^aData are means of 5 pooled leaves per plant, from four plants per treatment.

The treatments also affected the leaf concentrations of P, K, Ca, Mg, B, Mn and Cu. Namely P leaf concentration decreased with DFB, VN and SE for dilution, while plants treated with OG showed the highest concentrations, largely overcoming the optimal range thresholds indicated by Marschner [34] (Table 5).

Table 5. Leaf nutrient concentrations measured during fruit setting (9 June) and before harvest (September 2) of the second year. Data are means from 5 pooled leaves per plant, from four plants within each treatment. Treatment means followed by the same letter do not differ significantly from one another, according to Tukey's HSD tests (p = 0.05)

Optimal range	P (%)		K (%)		Ca (%)		Mg (%)		B (mg/kg)		Fe (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	0.18-0.30		1.1-1.5		1.3-2.2		0.2-0.35		30-50		n.a.		35-100		5-10		20-50	
Fertilizer/ Amendment	June 9	Sept 2	June 9	Sept 2	June	Sept	June	Sept	June	Sept	June	Sept	June	Sept	June	Sept	June	Sept
Control	0.3 b	0.6 b	2.0 b	2.0 b	1.0 ab	1.4 ab	0.3 a	0.2 c	34.1 a	42.0 b	193	109	21.5 b	17.8 c	7.7 c	7.8c	10.4	9.5
SMC	0.2 c	0.2 cd	1.8 b	1.6 c	1.2 a	1.5 a	0.3 a	0.3 a	23.0	29.0 d	141	99	26.7 ab	29.9 a	9.0 b	11.6 a	11.1	10.0
VN	0.1 d	0.2 d	2.2 a	2.0 b	0.9 b	1.3 b	0.2 b	0.2 c	20.3 c	31.3cd	158	87	25.2 ab	24.7 ab	9.6 a	10.6 ab	10.6	9.9
SE	0.2 c	0.3 c	2.2 a	2.1 b	1.2 a	1.5 ab	0.2 ab	0.2 b	23.3 b	32.4 c	146	92	29.9 a	26.1 ab	9.1ab	9.3bc	10.6	9.4
OG	0.4 a	0.8 a	2.3 a	2.5 a	1.0 ab	1.5 a	0.2 ab	0.2 bc	36.2 a	46.1 a	181	99	21.1 b	20.7 bc	7.6 c	9.1bc	10.5	9.7
SEM	±0.04	±0.13	±0.10	±0.14	±0.05	±0.05	±0.01	±0.03	±3.2	±3.3	±16.2	±3.8	±1.6	±2.1	±0.4	±0.7	±0.1	±0.1

^{a)}Apple optimal range thresholds as indicated by Marschner (1995).

SEM: standard error of the means.

The shoot growth rate differed among treatments when expressed as RGRs from June to August. Shoot elongation was significantly lower for the plants supplied with OG and for the control plants compared to the other treatments. Differences were mostly evident in July ($p = 0.0007$) and August ($p = 0.0062$), however, they became insignificant later on the season ($p = 0.71$) (Fig. 1A).

The control also showed a significantly reduced trunk growth compared to the other treatments. For the plants treated with OG trunk growth rate was intermediate between the control and the other treatments without showing significant differences from untreated plants (Fig. 1B).

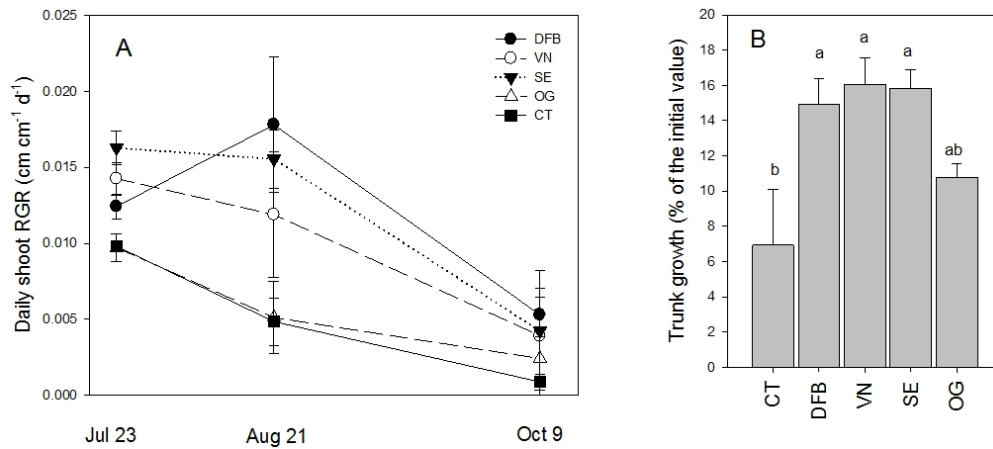


Fig. 1. A) Daily shoot elongation rates from July 23 to October 9 of the second year, expressed as the RGR. Within each single date, the differences among the nutrient treatments were significant for July 23 ($F = 0.0007$) and August 21 ($F = 0.05$), but not for October 9 ($F = 0.71$). B) Trunk caliper increments from June 25 to October 17 of the second year, expressed as percentages of the initial values recorded on June 25. All data refer to 16 plants per treatment (\pm SE). The treatments labelled with different letters are significantly different, according to Tukey's HSD test ($p = 0.05$).

The application of DFB was the only one inducing a significant increase of the numbers of flower clusters per plant as counted in April compared to Control (Fig. 2B). The number of fruit per plant in May was significantly greater for DFB compared to Control. The benefits over the control persisted also after the June drop (second fruit counting in July) for the plants treated with DFB and became significant for VN (Fig. 2B).

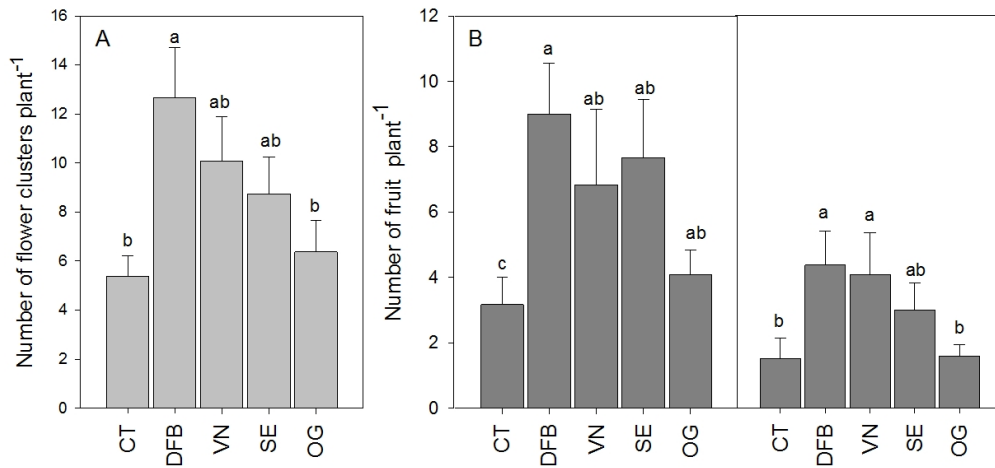


Fig. 2. A) Number of flower cluster per plant as measured in April. B) Number of fruit per plant, as measured on May 23 and July 15 of the second year. Data are means ±SE and refer to 16 plants per treatment. Within each dataset, the treatments labelled with different letters are significantly different, according to Tukey’s HSD test (p = 0.05).

3.3 Root Biomass and Morphology

VN, DFB and SE stimulated significantly higher root development in terms of total dry weight, as compared to both the control and the OG treatment (Fig. 3). Root dry weight was positively correlated with mineralized N in June ($r^2= 0.76$) and July ($r^2= 0.80$) (Fig. 4). The proportion, in weight, represented by the fibrous fraction of the total root weights, 6 months after the last fertilizer or amendment application, did not differ statistically between the treatments (data not shown). The maximum root length per plant did not differ statistically among the treatments, except for a significant increase for the OG treatment (Fig. 3B).

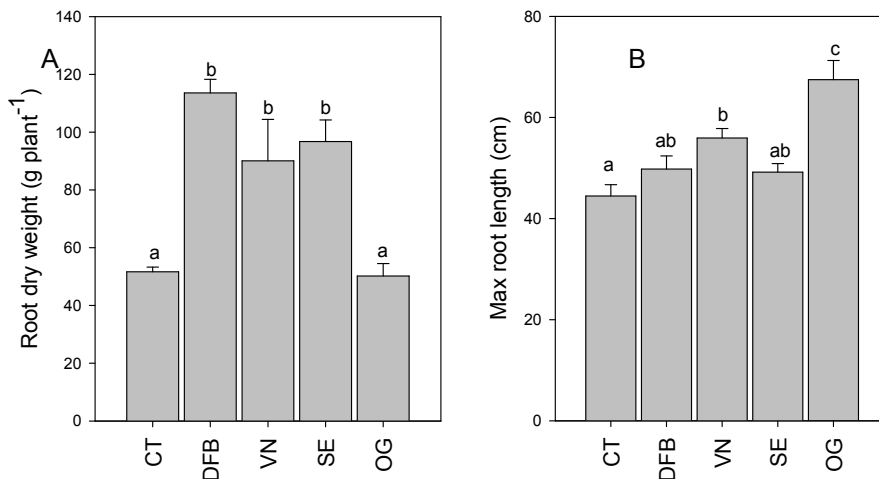


Fig. 3. A) The total root dry weight (g) per plant measured. B) The length of the longest root. The data refer to four plants per treatment (±SE) at the end of the second year. Treatments labelled with different letters are significantly different, according to the Tukey’s HSD test (p = 0.05).

When the root branching frequency was analyzed for single root orders, treatments showed similar branching frequencies on 3rd and 4th root order (data not shown); however, for the 5th order roots, there was a relevant decrease in the branching frequencies of plants treated with VN and OG. Branching frequency for 5th order roots was negatively correlated with soil salt concentration as measured in July ($r^2=0.82$) (Fig. 4). Differences on root diameter and SRL due to treatment were not significant (data not shown).

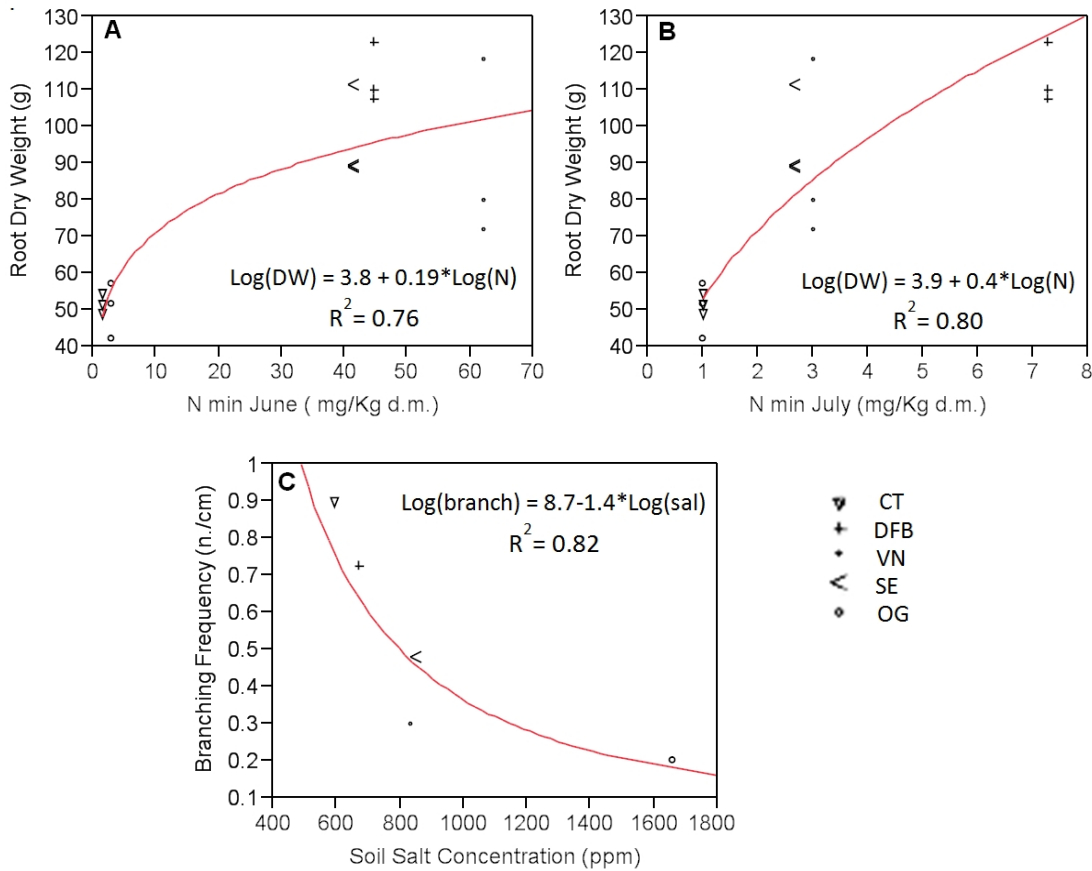


Fig. 4. Root dry weight as correlated with N mineralized in A) June and B) July. C) Branching frequency of fifth order root as correlated with soil salt concentration measured in July. Data refers to the second year. Root weight was measured on four plant per treatment, N mineralized and soil salinity were measured on four replicates per treatment plotted together. Branching frequency values represent the average of 30 root per plant per treatment.

4. CONCLUSION

Despite their very heterogeneous composition DFB, VN and SE showed a high N mineralization rate in the early stage of the experiment and generally promoted plant above and below ground development and enhanced the final number of fruit. The low N release rate of OG resulted insufficient in stimulating the performance of any vegetative or reproductive parameter compared to the control. The lack of a beneficial effect for OG may

be also partially ascribed to the early higher soil salt concentration. Analysis performed on root development indicated a reduced root branching frequency for 5th order roots. The branching frequency is a practical parameter for quantifying plant architectural modifications as a reaction to changes that occur in the surrounding environment. Root systems tend not to colonize soil where there is potential phytotoxicity either due to increased salinity or allelopathic molecules [35] and both: the dry weight of root and the number of root branches had been found to decrease on unsuitable growth condition [36,37]. A previous study on fine roots indicated a half-life of 240 days for root structures of 0.71 mm diameter [38]. It is consequently reasonable to consider that, in our study, the fifth order roots (diameter: 3.02 ± 0.09 mm) would survive through the 18 months of the trial and they can be a useful indicator for the immediate effects of treatments application, including stress due to salinity excess. The hypothesis of a temporary salinity stress is also supported by the greater tendency of the main roots to migrate in the presence of an unsuitable environment [39,40]. Thus, in the present study, was observed on the plants treated with OG, which roots presented the highest length values. The soil salt concentration measurements confirmed salinity as a potential cause of stress, since soil EC temporary exceeded the optimal thresholds and recovered later on the season.

The OG branching rate was not significantly different from other treatments on the younger roots (i.e., 3° and 4° order roots). Those root were probably not present when the amendment was applied, considering that the average apple fine root lifespan does not exceed 30 days [41]. The recovery of regular branching pattern following a period of non-uniform development was confirmed by the lack of difference in the final incidence of the fibrous root fraction on total root weight. This response indicates that stresses have been, finally, overcome and the environment has again become suitable for uptake.

In conclusion, all tested fertilizers and amendments acted to increase root and shoot development as well as the final yield, when compared with untreated plants. The only application of OG did not improve plant performances in comparison with the control. The OG was characterized by a low N release rate and a source of root stress identified as high salinity in this study. Although the current experience does not exclude the possibility of other causes for phytotoxicity due to OG supply. The study indicated how the use of oil mill and vine waste as amendment should be scheduled to be well in advance of the plant nutrient absorption peak, in order to allow a greater N release. Furthermore, the quantity of mill and vine waste supplied to the growing media should be moderated in order to reduce the risk of soil salinity excess and root stresses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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