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Soil and plant nutrition

Changes in leaf nutrient content and quality of pear fruits by biofertilizer application in northeastern Italy

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Abstract-The aim of this study was to verify the influence of biofertilizer application resulting from energy production from corn biomass on nutrient uptake by pear plants during the growing cycle, and on fruit quality. The experiment was carried out on a Siltic Haplic Calcisol in the Italian province of Ferrara, in a medium-density Abbé Fétel commercial orchard. Treatments consisted of control (no application) and biofertilizer (30 m^3 ha^{-1} biofertilizer application on the row), with four replicates. The following variables were evaluated: mineral N, microbial biomass and respiration in soil; nutrient content in leaves; and fruit quality. Biofertilizer application increased soil mineral N availability; soil microbial biomass and respiration, but the content of this nutrient did not increase in leaves. Leaf nutrient concentration varied during growth season and biofertilizer application increased potassium, phosphorus and zinc concentration in mature leaves and reduced leaf magnesium and manganese concentration. Biofertilizer application reduced fruit dry matter content, total soluble solids and boron concentration, with no effect on fruit firmness and titratable acidity. Biofertilizer application has positive effect on soil mineral N dynamics and soil microflora, altering the content of nutrients in leaves, favoring fruit production.

Index terms: Soil nitrate. *Pyrus communis*. Organic fertilization. Biodigester effluent. Corn biomass biofertilizer.

Alteração do teor foliar de nutrientes e qualidade dos frutos de pera pela aplicação de biofertilizante no nordeste da Itália

Resumo-O objetivo foi verificar a influência da aplicação de biofertilizante resultante da produção de energia a partir de biomassa de milho na absorção de nutrientes por plantas de pera durante o ciclo de cultivo, e na qualidade dos frutos. O experimento foi realizado em Ferrara (Itália), num pomar da cv. Abate Fétel, sobre Calcisolo Síltico Háplico. Os tratamentos consistiram em controle (sem aplicação) e biofertilizante (aplicação de 30 m^3 ha^{-1} de biofertilizante, localizada na fila), com quatro repetições. Foram avaliados: N mineral, biomassa e respiração microbiana no solo; teor de nutrientes em folhas; e parâmetros de qualidade dos frutos. A aplicação de biofertilizante aumentou a disponibilidade de N mineral, a biomassa e respiração microbianas, e mesmo assim o teor deste nutriente não aumentou nas folhas. Os teores dos nutrientes foliares variaram durante a estação de crescimento e a aplicação de biofertilizante aumentou os teores de potássio, fósforo e zinco nas folhas maduras; enquanto reduziu o teor de magnésio e manganês foliar. A aplicação de biofertilizante reduziu o teor de massa seca, de sólidos totais e de boro dos frutos, sem efeito na firmeza e na acidez titulável. O biofertilizante teve efeito positivo sobre a dinâmica do N mineral no solo e a microbiota edáfica, alterando o teor de nutrientes nas folhas, favorecendo a produção de frutos.

Termos de indexação: Nitrato no solo. *Pyrus communis*. Adubação orgânica. Resíduo de biodigestor. Biofertilizante de biomassa de milho.

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Introduction

Nitrogen fertilization is an important tool to increase orchard yield (CARRANCA et al., 2018). However, the importance of this nutrient has led to a great increase in fertilizer use; for example, in 2010, global use was more than one hundred million metric tons of nitrogen fertilizer. Furthermore, analyses based on past trends, methods, and practices estimate that a 170% increase in nitrogen fertilization might be required to double global food production by 2050 (TILMAN; CLARK, 2015).

However, it is known that the excessive application of mineral and synthetic fertilizers in intensive farming systems has led to nutrient accumulation in soils and groundwater, which is responsible for a decrease of soil organic matter (OM) (SVANBÄCK et al., 2019; OMARA et al., 2019). Nardi et al. (2004) verified that only farmyard manure fertilization maintained total organic carbon level of 40 t C ha^{-1}, measured in topsoil layers at the start of a 40-year experiment, while the average total organic C depletion was 43% with mineral fertilizers. In a rice-wheat system, farmyard manure application at 20 t ha^{-1} showed, after a period of 32 years, higher organic carbon concentration of 17% compared with NPK fertilizers in the 0–15 cm soil layer (KUKAL et al., 2009). In addition, losses of N and phosphorus (P) may decrease the water quality of rivers and lakes through the eutrophication process, resulting in an important problem (VOLK et al., 2009; NIE et al., 2018).

In this context, an alternative to the use of synthetic or mineral fertilizers is the application of organic materials as nutrient source. Increased soil organic matter (OM) content plays an important role in long-term soil fertility preservation, due to the improvement of its physical, chemical and biological properties (HAYNES, 2005; DĘBSKA et al., 2016; OLDFIELD et al., 2018; CIHANGIR; OKTEM, 2019). However, most agricultural soils of the Eastern part of the Po Valley in Italy have shown OM concentration often lower than 1.5% (UNGARO et al., 2002), due to the reduced availability of organic fertilizers and increasing farm specialization.

Thus, there is growing interest in the use of biodigester effluents (known as biofertilizers) in agriculture to provide nutrients as an alternative to mineral fertilizers (RAHEEM et al., 2016; DĘBSKA et al., 2016; VERONEZE et al., 2019). They are byproducts of the anaerobic digestion of organic materials, whose major purpose is the production of electricity and/or heat through combustion of the main product, biogas (TAMBONE et al., 2009; OLIVEIRA et al., 2011). These products have all the necessary elements for plant nutrition, varying concentrations according to the preparation method and the originating material (MARROCOS et al., 2012). In a literature review, Möller and Müller (2012) highlighted values ranging from 1.2-11.5, 1.2-9.1, 0.4-2.6, 1.0-2.3, 0.3-0.7 and 0.2-0.4 kg Mg^{-1} fresh matter for total K, total N, total P, total Mg and total S in residual anaerobic digestion product. Veroneze et al. (2019) verified that micronutrient concentrations are quite significant for plant fertilization, and the biofertilizer evaluated is a good alternative for the supply of Cu, Zn, Fe and Mn for plants. However, there is lack of information about its use in well-established plantations.

Based on the above, the aim of this study was to verify the influence of biofertilizer application resulting from energy production from corn biomass on nutrient uptake by pear plants during the growing cycle and on fruit quality.

Materials and methods

Experimental site characteristics

This study was carried out in 2014 in a commercial farm in the province of Ferrara (44º48'03 "N; 11º39'02"E), in the Emilia-Romagna region, northeastern Italy. The local climate can be defined as Cfb-humid temperate, according to the Köppen system updated by Peel et al. (2007). According to the survey carried out by the Servizio Geologico, Sismico e dei Suoli (2013), the local soil is a Siltic Haplic Calcisol in the FAO classification (1988). It presented the following characteristics (in the 0-40 cm depth): Sand: 26%; Silt: 46%; Clay: 28%; $pH_{(H2O)}$ 7.6; Organic Carbon: 14 g dm^{-3}; Total N: 1.8 g dm^{-3}; Olsen P: 22.4 mg dm^{-3}; K: 0.5 $cmol_c$ dm^{-3}; Ca: 13.0 $cmol_c$ dm^{-3}; Mg: 2.8 $cmol_c$ dm^{-3}; H+Al: 0.0 $cmol_c$ dm^{-3}; CEC (Cation Exchange Capacity): 16.3 $cmol_c$ dm^{-3}; SOB (Sum of Bases): 16.3 $cmol_c$ dm^{-3}; BS (Base Saturation): 100%.

The experimental field was a 12-year old European pear orchard (*Pyrus communis*L.) Abbé Fétel cultivar grafted onto Farold®40 rootstock. Spacing was 3.8 m between rows and 1.5 m in the row, amounting to 1,754 plants ha^{-1} (medium density). The training system was in free palmette. The orchard was equipped with a drip irrigation system. Soil was kept covered throughout the year by spontaneous vegetation, controlled by periodic cuts. Integrated production was adopted, following the rules of the Regione Emilia-Romagna (2013).

Experimental design and biofertilizer characteristics

The experimental design was completely randomized, consisting of two treatments with four replicates each. Each plot had 20 plants. Treatments were: control (no application) and biofertilizer (30 m^3 ha^{-1} application of biodigester effluent).

The liquid biofertilizer used in this experiment was provided by Palmirano Biogas Società Agricola (Ferrara, Italy), and was a residue of the anaerobic digestion of corn biomass to produce biogas. Biofertilizer was applied on June 2nd, 2014, when pear fruits had about 30 mm in diameter using a liquid organic fertilizer distributor, locating the product on a strip about 1 m in width (0.5 m on each side) along the row. The product amount to be applied was defined according to limits set in the rules for integrated production (REGIONE EMILIA-ROMAGNA, 2013). No other fertilizer was applied during the season.

The biofertilizer main characteristics can be verified in Table1. It is relatively rich in N, presenting considerable amounts of other macronutrients and micronutrients, and low concentration of heavy metals - barium (Ba), chromium (Cr), lead (Pb), cobalt (Co), and cadmium (Cd).

Table 1 - Chemical characteristics of biofertilizer and total applied by element.

Parameter[1]	Unit	Result	Total (kg ha^{-1})	Parameter	Unit	Result	Total (g ha^{-1})
pH	-	7.8	-	Zn	mg kg^{-1}	243.2	591
Dry matter	%	8.1	2,430	Mn	mg kg^{-1}	186.7	454
C	g kg^{-1}	375.0	911	Cu	mg kg^{-1}	120.6	293
N	g kg^{-1}	31.3	76	Ba	mg kg^{-1}	36.9	90
Ca	g kg^{-1}	16.4	40	B	mg kg^{-1}	31.7	77
P	g kg^{-1}	8.4	20	Cr	mg kg^{-1}	13.7	33
Mg	g kg^{-1}	7.8	19	Ni	mg kg^{-1}	7.9	19
K	g kg^{-1}	7.4	18	Cl	mg kg^{-1}	6.4	16
S	g kg^{-1}	3.8	9	Mo	mg kg^{-1}	6.4	16
Na	g kg^{-1}	2.4	6	Pb	mg kg^{-1}	4.7	11
Fe	g kg^{-1}	1.7	4	Co	mg kg^{-1}	0.5	1
Al	g kg^{-1}	1.0	2	Cd	mg kg^{-1}	0.4	1

[1]Source: Laboratory of Biochemistry, Department of Agricultural Sciences, University of Bologna (2014).

Soil analysis

Mineral N (ammonium - NH_4^+-N and nitric- NO_3^--N) soil concentration was measured through periodic sampling (1, 8, 30, 90, 120, 150 and 180 days after biofertilizer application -DAA) in the profile from 5 to 60 cm in depth (5 to 30 cm and 31 cm to 60 cm). The first 5 cm were discarded to eliminate crop litter interference in the analysis (BOONE et al., 1999).

Soil samples were homogenized and sieved (2 mm mesh). About 100 mL of a 2 mol L^{-1} potassium chloride (KCl) extractive solution was added to 10 g moist soil samples. After stirring for 1 h at 110 rpm and filtering, the resulting solution was frozen at -20 °C for storage until further analysis. NO_3^--N and NH_4^+-N concentrations were measured using automatic continuous flow analyzer (AA-3 Auto Analyzer; Bran + Luebbe, Norderstadt, Germany). Soil sub-sample was oven-dried at 105 °C until constant weight to determine moisture content.

Soil mineral N availability (in kg ha^{-1}) was estimated considering depth from 5 to 60 cm; width of 0.5 m on each side of the row under plants (therefore, it was not calculated on the total area, only in the row); and soil bulk density of 1,400 kg m^{-3}. This value is common to the region, as cited by Ventura et al. (2013), who analyzed similar soil.

Microbial biomass and respiration analyses were performed as described by Anderson and Domsch (1978), adapted from Jenkinson and Powlson (1976). Soil samples from 5 to 15 cm in depth were taken from plot in three dates: Oct. 2nd (120 DAA), Nov. 4th (150 DAA) and Dec. 10th, 2014 (180 DAA). After being homogenized and sieved to remove plant debris and other materials, 50 g of moist soil from each sample were placed in glass jars and left under damp cloth overnight to standardize relative humidity inside jars. On the following day, 200 mg of D-glucose were added to each sample, which were then homogenized and hermetically sealed at equal three-minute intervals. After incubation for three hours, the carbon dioxide (CO_2) concentration in the jar head space was read using infrared CO_2 analyzer EGM-4 (PP Systems, Amesbury, USA). The following data have been recorded: initial peak ($t_0 = 0$ min), lowest value ($t_1 \approx 1$ min) and value presented 1 min after the previous one ($t_2 \approx 2$ min) at regular 3-min intervals. Thereby, the difference between t_1 and t_2 (ΔCO_2) was obtained, corresponding to the amount of CO_2 produced by microorganisms in the considered time. Regression equation was then used to calculate the CO_2 rate produced per unit of time (in ppm CO_2 h^{-1}) and the amount of microbial biomass (in µg C g^{-1} of soil), according to Anderson and Domsch (1978).

Plant analysis

From each plot, samples of 20 mature, expanded, and healthy leaves were taken from shoots on the middle third of the canopy in July (summer - mature leaves, 30 DAA) and October (autumn – before senescence started, 120 DAA). After this procedure, nets were placed around a few branches in each plot to enable the collection of leaves during their natural abscission in November and December (winter – post-abscission, 150 and 180 DAA). Total leaf area was measured using portable area meter (Li-3000, LiCorInc., Lincoln, Nebraska). Leaves were then washed in HCl (0.1 mol L^{-1}) and surfactant (Tween 20®) (0.1%) solution according to Álvarez-Fernández et al. (2001), rinsed in tap and distilled water, oven-dried at 60 °C until constant weight, weighed, and milled. Each sample was mineralized according to US EPA Methods 3052 (KINGSTON, 1988): sub-samples weighing 0.5 g were placed in special containers added of 8 mL of 65% nitric acid (HNO_3) and 2 mL of 30% hydrogen peroxide (H_2O_2). Once sealed, containers were placed in microwave lab station (Ethos TC, Milestone, Bergamo, Italy) at 180 °C for 20 min. Then, samples were analyzed using optical emission spectrophotometry with inductively coupled plasma (ICP) (Ametek Spectro, Arcos, Kleve, Germany) to determine the following nutrients: P, K, Ca, Mg, S, Cu, Zn, B, Mn and Fe. N concentration was obtained by the Kjeldahl method, adapted by Schuman et al. (1973). Dry leaf sub-samples weighing 0.5 g were mineralized with 14 mL of 95:5 (v/v) sulfuric acid (H_2SO_4 95%) and phosphoric acid (H_3PO_3 85%) solution, at 420 °C for 3 h; distilled with 32% (v/v) sodium hydroxide (NaOH) and titrated with 0.2 mol L^{-1} HCl.

Commercial harvest was on September 3rd, 2014 (90 DAA); total yield per treatment was measured, and fruits that reached base diameter of 65 mm or more were considered commercial. For each plot, samples of 16 fruits were collected, weighed, and submitted to the following qualitative assessments:

- Fruit firmness: measured using penetrometer with 8-mm plunger (Effegi, Ravenna, Italy), and expressed in kg;

- Total soluble solids (TSS): measured using portable digital refractometer PR-1 (Atago, Tokyo, Japan) and expressed in °Brix;

- Titratable acidity: measured using automatic titrator Compact Titrator (Crison, Barcelona, Spain), and expressed in g L^{-1} of malic acid equivalent.

Pulp dry matter content was obtained removing the peel and oven-drying sub-samples at 65 °C until constant weight. To obtain fruit mineral concentration, fruit sub-samples were lyophilized, milled, mineralized and analyzed according to methods previously described for leaf analysis.

Data were submitted to analysis of variance using the SAS/STAT statistical software (Cary, USA), and the SNK test (Student-Newman-Keuls, $p = 0.05$) for separation.

Results and discussion

Soil analysis

Soil moisture content was higher with biofertilizer application in five of the seven dates throughout the monitoring period (Figure 1A). Soil moisture in treated plots was 22% higher than control at 150 DAA, and 14% higher at 180 DAA, indicating that this was a long-lasting effect. This result may be due to the improvement in water retention with biofertilizer application, as verified by Alencar et al. (2015). In addition, the mulch effect of the biofertilizer may have contributed to this increase in moisture, locally decreasing evaporation rate and contributing to save soil water, which is important mainly because it is an irrigated area (LAL, 2009; JORDÁN et al., 2010). This effect is probably not due to the increase in inorganic matter content, since successive applications would be necessary, and in this case only one application was carried out (LOURENZI et al., 2016). Soil NO_3^--N concentration increased by biofertilizer application, and this effect was rapid, since it was already detected at 8 DAA (Figure 1B). Moreover, it proved to be lasting, because important differences were demonstrated up to 150 DAA.

In untreated soil, NO_3^--N concentration decreased during the intensive shoot growth phase, when plant demand for N is high (June and July - 8 and 30 DAA). The increased NO_3^--N concentration at 90 DAA may be due to mineralization of OM naturally present in the soil - enhanced by high temperatures and rainfall that occurred in the summer (130 mm of rain in July and August) - combined with low root uptake rate at that time (SUGAR et al., 1992; QUARTIERI et al., 2002).

NO_3^--N is mobile and susceptible to loss due to leaching due to its low adsorption to soil particles (NAZ; SULAIMAN, 2016). Therefore, biofertilizer application provided synchronization of N supply according to plant demand, avoiding mineral N accumulation in the soil, and consequently, risk of NO_3^--N leaching (DIACONO; MONTEMURRO, 2010).

Soil NH_4^+-N concentration did not increase during the evaluation period, except in analysis carried out at 180 DAA (Figure 1C). Probably, most NH_4^+ added to the soil by the biofertilizer was rapidly oxidized to NO_3^- through the nitrification process performed by chemosynthetic bacteria (DI et al., 2009; MINOGUE et al., 2012), contributing to increase the NO_3^- concentration in the soil solution. In addition, there may have been loss of NH_4^+-N by volatilization (loss to the atmosphere in

the form of ammonia gas - NH_3), which occurs when it is in solution at neutral or alkaline pH (ROCHETTE et al., 2013), as is the case of the experimental site (pH H_2O: 7.6).

Total mineral soil N availability was higher in treated plots, as compared to those untreated in almost all analyzed dates (Figure 1D). At 30 DAA, N availability was five times higher in treated plots, and almost double at 120 and 150 DAA, while at 180 DAA, this difference disappeared. However, the increase in mineral N availability was never equal to the amount of N provided by the biofertilizer, indicating that the nutrient remained in the 0-5 cm layer (not sampled) was absorbed by plants, lost by NO_3^- leaching, NH_3 volatilization, or denitrification.

Proper soil N availability during the growing season is important for the vegetative growth of pear trees. Although only about 10% of N allocated to fruits come from uptake in summer (June-August) (QUARTIERI et al., 2002), N absorbed at this time may be stored in the woody plant structures and remobilized the following spring for floral development (SUGAR et al., 1992), ensuring good budding and flowering in the following year.

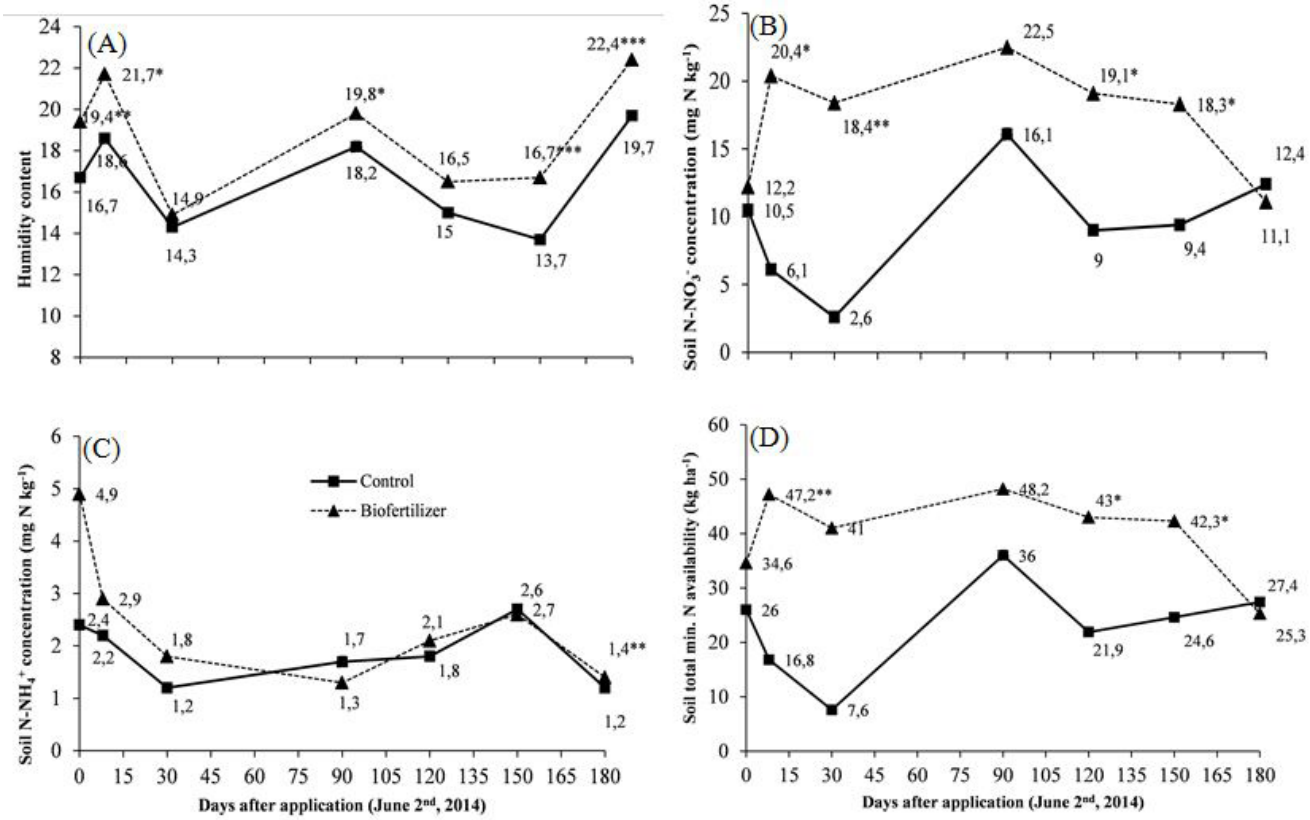

Figure 1. Effect of biofertilizer application on soil: 1A - Moisture content; 1B:NO_3^--N concentration; 1C: NH_4^+-N concentration; 1D: Total Mineral N availability up to 180 days after application (average of 5 - 30 cm and 31 - 60 cm depths for 1A, 1B and 1C). Soil N availability at 5 - 60 cm in depth.*, ** and ***: significant for p <0.05, p<0.01 and p <0.001, respectively.

Microbial respiration and biomass

The increase in soil N is linked to a consistent increase in microbial biomass, expressed by microbial C, and activity (shown by CO_2 produced in respiration), resulting from biofertilizer application (Table 2). This is due to the microbial biomass that acts as buffer of N in the soil, controlling the availability of this nutrient by means of mineralization and immobilization (BARRETO et al., 2008). Considering that the soil microflora feeds on and is part of OM, the addition of biofertilizer provided conditions to increase its metabolic activity and population. This effect was observed in all analyzed dates; in early fall (120 DAA), microbial biomass was 68% higher in treated soil as compared to control, whereas microbial activity was 60% higher and at the end of the trial (180 DAA), differences were respectively 64% and 62%.

Table 2. Effect of biofertilizer application on soil microbial activity (5 – 15 cm depth).

Treatment	Oct. 2nd 2014 120 DAA		Nov. 4th 2014 150 DAA		Dec. 10th 2014 180 DAA	
	Produced CO_2 ppm CO_2 h^{-1}	Microbial C µg C g^{-1}	Produced CO_2 ppm CO_2 h^{-1}	Microbial C µg C g^{-1}	Produced CO_2 ppm CO_2 h^{-1}	Microbial C µg C g^{-1}
Control	16	51	17	52	13	46
Biofertilizer[1]	26	86	22	72	21	75
Significance[2]	*	*	**	**	*	*

[1]Biofertilizer applied at 30 m^3 ha^{-1} dose on June 2nd 2014. [2] * and **: significant for p <0.05 and p<0.01, respectively.

Microbial C is an important soil quality attribute - acting as a nutrient reservoir and central C-cycle compartment - and is one of the most sensitive to changes caused by soil management practices (GAMA-RODRIGUES; GAMA-RODRIGUES, 2008; BALOTA, AULER, 2011). Its increase indicates improvement in soil quality - since microbial C is closely linked to total soil organic C (data not presented) (DIACONO; MONTEMURRO, 2010). It should be noted that microorganisms are the main agents of nutrient mineralization, being that about 90% of nutrients are mineralized by microorganisms, making them available in the soil solution and, consequently, in plants (LAVELLE, 2000).

Leaf analysis

Leaf area and dry weight, in both treatments, were strongly reduced between autumn and winter. Leaf area was higher in the beginning of senescence (on average 21.6 cm^2 $leaf^{-1}$) than at post absission (14.9 cm^2 $leaf^{-1}$), as it was observed for leaf dry weight, which decreased from 241 to 156 mg $leaf^{-1}$ (-35%). Meanwhile, specific leaf weight (SLW) remained unchanged (on average 10.8 µg cm^{-2}). In deciduous tree species, SLW shows initial increase with increasing leaf age until completion of leaf structural differentiation and then remain constant or moderately increases or decreases with further increases in leaf age (NIINEMETS, 2016). These variations in leaf dry mass per area unit (SLW) and nutrient content per dry mass affect the amount of nutrient removed by the tree canopy.

In both treatments, nutrient concentration in mature pear leaves (Table 3), with exception were Fe and Cu, was mostly within the normal range expected for the season for Abbé Fétel grown in Emilia-Romagna (TOSELLI et al., 2002). According to Toselli et al. (2002), the optimal Fe and Cu concentration in mature pear leaves is 60-95 mg kg^{-1} and 25-50 mg kg^{-1}, respectively. Thus, Fe concentration was lower (33 mg kg^{-1} - control and 39 mg kg^{-1} - biofertilizer) and Cu concentration was much higher than expected (118 mg kg^{-1} - control and 121 mg kg^{-1} - biofertilizer) (Table 3). K concentration was below recommended values only in the control treatment, indicating the need for complementation with fertilization, despite the high soil K content (0.5 $cmol_c$ dm^{-3}).

Table 3. Effect of biofertilizer application on leaf mineral concentration in three different phenological stages, and on pear fruit mineral concentration at commercial harvest (90 days after application).

Treatment	Macronutrients (g kg^{-1} dry matter)						Micronutrients (mg kg^{-1} dry matter)				
	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Mature leaves (July 2nd 2014) – 30 DAA											
Control	21.8	1.3	5.5	11.6	3.6	1.1	33	34	118	30	23
Biofertilizer[1]	22.3	1.4	7.5	12.1	2.9	1.2	39	22	121	57	22
Significance[2]	ns	*	**	ns	*	ns	ns	**	ns	**	ns
Initial leaf senescence (Oct. 2nd 2014) – 120 DAA											
Control	19.6	1.4	5.2	15.7	3.3	1.3	52	32	222	77	29
Biofertilizer[1]	21.1	1.4	6.6	16.3	2.7	1.3	46	16	205	79	28
Significance[2]	ns	ns	ns	ns	*	ns	*	**	*	ns	ns
Post-abscision leaves (Nov. 5th and Dec. 10th 2014) – 150 and 180 DAA											
Control	13.8	1.1	5.4	17.7	3.4	1.2	169	39	251	77	37
Biofertilizer[1]	14.3	1.2	8.5	18.8	2.7	1.2	140	18	260	92	39
Significance[2]	ns	ns	*	ns	**	ns	ns	***	ns	ns	ns
Commercial fruit (Sept. 3rd 2014) – 90 DAA											
Control	2.62	0.38	3.36	0.30	0.25	0.20	6	1	21	4	27
Biofertilizer[1]	2.77	0.45	4.05	0.32	0.29	0.20	5	1	26	5	14
Significance[2]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**

[1]Biofertilizer applied at 30 m^3 ha^{-1} dose on June 2nd 2014. [2]ns, *, ** and ***: not significant, significant for p <0.05, p <0.01 and p <0.001, respectively.

Although leaves were thoroughly washed prior to analysis, the high leaf Cu concentration is most likely derived from the continued use of cupric fungicides in the control of diseases (REGIONE EMILIA-ROMAGNA, 2013), leading to leaf Cu uptake. These treatments are performed even during leaf senescence to prevent pathogens from penetrating the abscission septa. Toselli et al. (2002), also in Emilia Romagna conditions, recorded leaf Cu and Zn concentrations reaching up to 500 mg kg^{-1} each in Conference and Abbé Fétel pears during summer.

Excess Cu can prevent Fe absorption and translocation to shoots, which could be an explanation for the low leaf Fe concentration found in this experiment (AZEEZ et al., 2015; ADREES et al., 2015). The mechanisms behind this antagonistic effect can be related to the saturation of negative charges of root apoplastic environment by Cu. Although a clear decrease of leaf Fe concentration as a response of increasing soil Cu concentration was found in grapevine (TOSELLI et al., 2009), this was not observed in pear, where increasing soil Cu application up to 1000 mg Cu kg^{-1} DW had no effect on leaf Fe concentration (TOSELLI et al., 2008). In addition, Fe deficiencies appear often in sub-alkaline pH (BRUMBAROVA et al., 2015), such as that of this study, as a consequence of Fe insolubilization.

Biofertilizer application increased leaf K, P and Zn concentration as compared to control (Table 3). This effect may have reduced Mg and Mn absorption due to antagonism caused by competitive inhibition derived from K and Zn absorption (TOSELLI et al., 2002). This behavior was evidenced by the strong negative correlation between K and Mg (-0.81) and moderate negative correlation between Zn and Mn (-0.65). No effect of treatments on leaf N, Ca, S and B concentration was observed.

Overtime, decrease in N concentration and increase in Ca, Fe, Cu and B concentration for both treatments during the growing season were observed. P, K, Mg and S concentrations were stable (Table 4). Zn concentration increased only in control, while its level remained stable (and relatively higher) in plants of treated plots. Similarly, Baldi et al. (2014), worked with nectarine trees, which belongs to the Rosaceae family, the same family of pear trees, reported, between mature stage and post-abscission, increase in P, Ca, Mg, Fe and Mn concentration per leaf area, and reduction in N, K and Zn concentration in the same period, while Cu concentration remained stable.

Table 4. Leaf nutrient concentration of 12-year old pear trees according to the phonological stage.

Phenological stage[1]	Macronutrients (g kg^{-1} dry matter)						Micronutrients (mg kg^{-1} dry matter)				
	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control											
Mature leaves	21.8 a	1.3	5.5	11.6 b	3.6	1.1	33 b	34	117 b	30 b	23 b
Initial leaf senescence	19.6 a	1.4	5.2	15.7 a	3.3	1.3	52 b	32	222 a	77 a	29 ab
Post-abscision leaves	13.8 b	1.1	5.4	17.7 a	3.4	1.2	169 a	39	251 a	77 a	37 a
Significance	***	ns	ns	***	ns	ns	**	ns	**	***	**
Biofertilizer[2]											
Mature leaves	22.3 a	1.4	7.5	12.1 b	2.9	1.2	39 b	22 a	121 b	57	22 b
Initial leaf senescence	21.1 a	1.4	6.6	16.3 a	2.7	1.3	46 b	16 b	205 a	79	28 ab
Post-abscision leaves	14.3 b	1.2	8.5	18.8 a	2.7	1.2	140 a	18 ab	260 a	92	39 a
Significance	***	ns	ns	**	ns	ns	***	*	**	ns	**

[1]Sampling dates: mature leaves: July 2nd 2014 – 30 DAA; initial senescence leaves: Oct. 2nd 2014 – 120 DAA; post-abscission leaves: average of samplings performed on Nov. 5th and Dec. 10th 2014 – 150 and 180 DAA. [2]Liquid biofertilizer applied at 30 m^3 ha^{-1} dose on June 2nd 2014. [3]ns, *, ** and ***: not significant, significant for p <0.05, p <0.01 and p <0.001, respectively. Averages followed by the same letter are not statistically different.

N concentration reduction reflects nutrient translocation to storage organs (branches, stem and roots) at the end of the growing season, since during senescence, the transport of soluble nutrients is facilitated (QUARTIERI et al., 2002; ENGELS et al., 2012). Leaf remobilization in late summer/autumn in pear is high for N, K, P, and Zn (QUARTIERI et al., 2002; NETO et al., 2008). The increase in leaf Ca and micronutrient concentration is a result of their low mobility in the plant, while the possibility that plants have continued to uptake these nutrients during fall/winter should not be disregarded, consequently, their leaf concentration increased (ENGELS et al., 2012).

Yield and fruit quality

Considering the average of treatments, fruit yield increased by 28% in plants treated with biofertilizer compared to control (5.9 t $ha^{-1)}$ (Table 5). The percentage of commercial fruits remained stable (72% and 73%), regardless of treatment. Fruit firmness and titratable acidity were not affected by treatments while a slight reduction in total soluble solids (TSS) and dry matter was observed in fruits from plants treated with biofertilizer (Table 5). TSS values are similar to those observed by Sorrenti et al. (2012), but their reduction by the biofertilizer application makes fruits less sweet, which could negatively impact their organoleptic quality. The average larger fruit production may have led to the dilution of sugars and other organic compounds, as also suggested by Lemiska et al. (2014) in strawberry and also verified by Amiri and Fallahi (2009) in apple submitted to different cow manure and poultry manure application rates.

Table 5. Effect of biofertilizer application on yield and fruit quality of 12-year old pear trees at commercial harvest (90 days after application).

Treatment	Yield		Fruit qualitative assessments			
	Total	Commercial (minimum ø 65 mm)	Firmness	TSS	Titratable acidity	Dry matter
	(t ha^{-1})	(t ha^{-1})	(kg)	(°Brix)	(g l^{-1} malic acid)	(%)
Control	20.8	14.4 (73%)	4.08	13.8	2.20	17.0
Biofertilizer[1]	26.7	19.1 (72%)	4.10	12.0	2.24	15.6
Significance[2]			ns	*	ns	*

[1]Biofertilizer applied at 30 m^3 ha^{-1} dose on June 2nd 2014. [2]ns and *: not significant and significant for p <0.05, respectively.

Regarding fruit nutrient concentration, there were no differences, except for B, which was lower in the biofertilizer treatment (Table 3). Adequate B content is important for membrane integrity and may help preventing browning disorders during post-harvest conservation (HERRERA-RODRÍGUEZ et al., 2010; GANIE et al., 2013); however, this effect was not verified for fruit firmness (Table 5). Large fruit production could explain the decrease in fruit B concentration in treated plants, as B suffered a dilution due to the high number of fruits. This is a hypothesis, in part supported by a previous study on Turkish Deveci pear that showed higher B concentration in fruit than in leaves due to the translocation of both soil and leaf-applied B to fruits (GÜREL; BAŞAR, 2016). This implies that the amount of B applied was not adequate for the orchard demand. In addition, in tomato, B deficiency altered B distribution with greater concentration in stem and petiole than in fruit cluster and leaves (CHOI et al., 2015).

Conclusions

Biofertilizer application resulted in increased soil N availability, in synchrony with plant demand, but without effect on leaf N content in the various phases of the growth cycle.

Biofertilizer application resulted in higher leaf K and Zn content, which caused reduction in Mg and Mn contents.

Changes in leaf nutrient concentrations promoted by biofertilizer application reduced the TSS of fruits, influencing their quality.

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