Nutrient removal by apple, pear and cherry nursery trees

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Abstract

Given that nursery is a peculiar environment, the amount of nutrients removed by nursery trees represents a fundamental acquisition to optimise fertilisation strategies, with economic and environmental implications. In this context, we determined nutrient removal by apple, pear and cherry nursery trees at the end of the nursery growing cycle. We randomly removed 5 leafless apple (Golden Delicious/EMLA M9; density of 30,000 trees ha^{-1}), pear (Santa Maria/Adams; density of 30,000 trees ha^{-1}) and cherry (Alex^{TM}/Gisela 6®; density of 40,000 trees ha^{-1}) trees from a commercial nursery. Trees were divided into roots (below the root collar), rootstock (above-ground wood between root collar and grafting point) and variety (1-year-old wood above the grafting point). For each organ we determined biomass, macro- (N, P, K, Ca, Mg, S,) and micro- (Fe, Mn, Zn, Cu, and B) nutrient concentration. Pear trees were the most developed (650 g (dw) tree^{-1}, equal to 1.75 and 2.78 folds than apple and cherry trees, respectively) whereas, independently of the species, variety mostly contributed (>50%) to the total tree biomass, followed by roots and then above-ground rootstock. However, the dry biomass and nutrient amount measured in rootstocks (including roots) represent the cumulative amount of 2 and 3 seasons, for Gisela® 6 (tissue culture) and pome fruit species (generated by mound layering), respectively. Macro and micronutrients were mostly concentrated in roots, followed by variety and rootstock, irrespective of the species. Independently of the tissue, macronutrients concentration hierarchy was N>Ca>K> P>Mg>S. Removed N by whole tree accounted for 6.58, 3.53 and 2.49 g tree^{-1} for pear, apple and cherry, respectively, corresponding to almost 200, 107 and 100 kg N ha^{-1}, respectively. High amounts of K and Ca were used by pear (130-140 kg ha^{-1}) and apple trees (~50 and 130 kg ha^{-1} of K and Ca, respectively), while ~25 kg K ha^{-1} and 55 kg Ca ha^{-1} were calculated for cherry nursery trees. Among micronutrients, Fe was the most required (~3 kg ha^{-1}) independently of the species. B removal ranged between 1.2 and 2.4 kg ha^{-1} (80, 40 and 30 mg tree^{-1} for pear, apple and cherry, respectively), whereas Mn, Cu and Zn accounted for few hundred g ha^{-1}, irrespective of the species. Given that nutrient concentration among tissues resulted within the same order of magnitude, irrespective of the species, differences in removal were mainly driven by the tree biomass as proved by the significant correlations between plant dry biomass with most of the nutrients we observed.

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Introduction

High-quality young trees represent an essential prerogative in commercial orchards (Kaplan and Baryla, 2006) in order to reduce the non-bearing phase. Fruit trees are usually commercially distributed after a two- three-year nursery growing cycle and graft is mostly adopted for trees designed for intensive orchards. Cultivars are frequently budded on late summer onto 1- 2-year-old rootstocks, which are in turn obtained by seeds, mound layering, trench layering, hardwood cutting or micropropagation. The following season, grafted trees are carefully managed in terms of irrigation and fertilisation to produce an upright leader provided by consistent lateral branches (Bielicki *et al.,* 2002). At the end of the nursery growth cycle, trees should be adequately developed with favorable size and root:shoot ratio. Additionally, young trees must accumulate high levels of reserves [carbohydrates and nitrogen (N)] to support the new growth until photosynthesis and mineral root uptake can sustain plant metabolism (Millard, 1995).

To ensure optimal growing conditions, fertilisation of maiden or grafted trees plays a crucial role in the nursery (Timmer and Aidelbaum, 1996; Malik and Timmer, 1998; Neto *et al.,* 2008). Nevertheless, nursery is a peculiar growth environment characterised by: i) sandy and poorly structured soils to limit root damages during mechanical tree removal; ii) ultra-high planting density (often >40.000 trees ha^{-1}) to best use soil surface and optimise the agronomic management (irrigation, fertilisation, pest and diseases control); iii) high competition among tree roots as a consequence of the high planting density and soil texture. As outcome, nursery trees are more dependent on the mineral uptake than mature trees because of their smaller root size, limited storage reservoirs and reduced vegetative growth (Bi *et al.,* 2004). Therefore, with the aim to promote a fast growth, generous fertilisation rates and irrigation volumes are sometimes adopted (Castle and Rouse, 1990) in commercial nurseries.

As a result, the amount of nutrients supplied during nursery phase may be in excess compared to the quantities effectively removed by crops, as described in a survey for forestry species recorded in North America, Britain and Germany (van den Driessche, 1980). Similarly to mature orchards, the amount of nutrients removed by different species in the nursery is a basic knowledge to optimise fertilisation strategies, as this has both economical and environmental implications. van den Driessche (1980) reported that amount of nutrients removed by conifer nursery crops ranged between 50 and 200 kg N ha^{-1}, 4 and 35 kg phosphorus (P) ha^{-1}, 25 and 105 kg potassium (K) ha^{-1}. However, information about nutrient removal of temperate perennial crops during the nursery growth period are still lacking.

The eastern Po valley (Italy) is a region traditionally well known for the importance of the nursery industry, which generates consistent income. For instance, over than 32 millions of woody fruit trees (including rootstocks and excluding berries) were produced in 2014 (Civi-Italia, 2015), mainly exported.

Therefore, the objective of this study was to quantify the mineral nutrient removal and partitioning among organs by apple, pear and cherry trees at the end of the nursery growth phase. These acquisitions may serve to optimise fertilisation strategies for the three species in temperate regions with potential economic and environmental positive implications.

Materials and methods

At the end of the growing nursery phase (winter, 2015), five leafless apple (cv. Golden Delicious *Malus domestica* B. grafted on M9 EMLA), pear (cv. Santa Maria *Pyrus communis* L. grafted on quince Adams *Cydonia oblonga* M.) and cherry (cv. Alex™ *Prunus avium* L. grafted on Gisela® 6 *Prunus cerasus* L. *x Prunus canescens* B.) trees were randomly removed from a commercial nursery (Salvi Vivai s.s.) located in the Italian eastern [Lagosanto (FE)] Po Valley (44° 80' N 12° 17' E; 0 m a.s.l.). The region has a temperate sub-continental climate with cold winters, humid and warm summers, mainly without dry seasons. The average temperature of the area is 23.4°C, rarely below -6°C or above 36°C. Yearly precipitations are generally below 800 mm, mostly concentrated in spring and autumn.

The nursery was open-field and the main soil physical and chemical characteristics are summarised in Table 1.

When removed, pear trees (1.80 m tall) were characterised by abundant and well-developed (>5) lateral branches, uniformly distributed along the central leader. Cherry trees consisted of a tall (>2 m) leader (~20 mm Ø), laterally devoid by any branch but covered with newly differentiated buds, as usually observed for young nursery sweet cherry trees in other environments (Moghadam and Zamanipour 2013). Apple trees were characterised by a ~1.7 m tall central leader with few (~1-3) and poorly developed lateral branches. In late summer (2014), trees were budded (Chip-budding technique) in the field at about 100-150 mm above the ground level and the planting distance was 0.33 m × 1 m, 0.33 m × 1 m and 0.25 m × 1 m (equal to densities of 30,000, 30,000 and 40,000 trees ha^{-1}) for apple, pear and cherry, respectively. Trees were irrigated by a one-line drip irrigation lying on the ground of each row from May through September with volumes up to 5 mm for each watering (full summer), according to the evapotranspiration rates. Approximately, yearly irrigation volumes were equal to 3000 m^3 ha^{-1}. Before the nursery establishment, the soil was amended with organic matter while during the growing season trees received N, P and K mineral inputs at rates of 120, 90 and 75 kg ha^{-1}, respectively. Microelements, mostly iron (Fe) were also periodically distributed. Fertilisers were supplied through fertigation. Weeds were mechanically removed at the beginning of the season, while herbicides were then used. Commercially available chemical pesticides were repeatedly sprayed over the tree canopy to control pests (*i.e.* pear psylla, aphids, codling moth, mites, pandemis *etc*.) and diseases (*i.e.* brown spot, apple scab, Monilinia, powdery mildew, bacterial canker, coryneum, *etc*.).

Harvested trees were divided into roots (below the root collar), rootstock (above-ground wood between root collar and grafting point) and variety (wood above the grafting point). While the latest organ was 1-year-old independently of the species, rootstocks (including roots) of cherry and pome fruit trees were 2 and 3-year old, respectively, as Gisela® 6 was obtained by tissue culture (*in vitro*) while M9 and quince Adams rootstocks were generated by mound layering.

Total fresh weight of each organ was recorded, then subsamples per tree were oven-dried at 65°C until constant weight and milled (<0.5 mm mesh) (Pulverisette 14, Fritsch GmbH, Idar-Oberstein, Gemany).

Macro [N, P, K, calcium (Ca), magnesium (Mg), sulphur (S)] and micro [Fe, manganese (Mn), zinc (Zn), copper (Cu) and boron (B)] nutrient concentration was determined on each individual tissue. N was determined by the Kjeldahl method (Schuman *et al.,* 1973) by mineralising 1.0 g (d.w.) with 10 mL of a 95:5 (v v^{-1}) sulphuric acid:phosphoric acid (H_2SO_4:H_3PO_4) mixture at 420°C for 180 min and subsequent distillation with 32% (v v^{-1}) sodium hydroxide (NaOH) and titration with 0.1 *N* H_2SO_4.

P, S, B and metals (K, Ca, Mg, Fe, Cu, Zn and Mn) were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES), (Ametek Spectro Arcos EOP, Kleve, Germany), after digestion with nitric acid (HNO_3) (Kingston, 1988) by a microwave lab station (Ethos TC-Milestone, Bergamo, Italy).

The amount of nutrients removed by organs was calculated multiplying the mineral concentration by the organ dry weight. The amount of minerals removed by each tree was obtained by adding the fractions of the three organs. Per each species, we estimated the amount of minerals removed by 1 ha of nursery, according to the relative tree density.

Statistical analyses

Coefficient of determination (R^2) between dry biomass and nutrient removed by whole trees was calculated using linear regression analysis. Data were submitted to analysis of variance using PROC MIXED with a compound symmetry covariance structure, according to a randomised experimental design with 5 replicates. When analysis of variance among the three species showed a statistical effect, means were separated by Tukey's honest significant difference test (at $P \leq 0.05$). Statistical analyses were performed by using SAS 9.0 software (SAS Institute Inc., Cary, NC, USA). Within nutrients, confidence interval at 95% ($_{95}$CI) was calculated for each means and represented in column.

Results

Young pear trees accounted for the highest wood canopy and root dry weight, whereas values between the other two species were statistically comparable (Figure 1). The dry weight of the apple rootstock was higher than cherry and pear trees and within

each species, the wood variety mostly contributed (>50%) to the total tree biomass, followed by roots and then above-ground rootstock (Figure 1). The pear root biomass was 2-time higher than the other species and the above:below the grafting point wood ratio was 1.25, 1.36 and 1.59 for cherry, apple and pear trees, respectively. Total pear tree biomass accounted for almost 650 g tree^{-1}, equal to 1.75 and 2.78 times that of apple and cherry trees, respectively. The latter species showed the lowest total dry weight, slightly over 230 g tree^{-1} (Figure 1).

Independently of the species, macro and micronutrients were overall mostly concentrated in roots, followed by variety and finally by the above-ground portion of the rootstock (Table 2). Only the concentration of Fe and, to a less extent Mn, was higher in rootstock than in the wood variety. With the exception of Ca in the rootstock, N was the most concentrated element in all tissues, followed by Ca, K, P, Mg and S (Table 2). The concentration of N was highest in cherry roots whilst those of Mg and K were higher in tissues of pear trees (Table 2). Among micronutrients, Fe was the most abundant, followed by B, Mn, Cu and Zn, irrespective of the organ and species (Table 2).

Within species, the amount of macronutrients removed followed this order: N>Ca>K>P>Mg>S (Figure 2). Pear trees removed higher amounts of macronutrients compared to cherry and, with the exception of Ca and S, to apple. Unless for N and Mg, apple removed higher amounts of nutrients than cherry (Figure 2). In details, the amount of N removed by whole trees was in average 6.58, 3.53 and 2.49 g for pear, apple and cherry, respectively (Figure 2). Considerable amounts (> 1 g tree^{-1}) were also calculated for Ca and K, while less than 1 g tree^{-1}was measured for P, Mg and S, independently of the species (Figure 2).

Within micronutrients, removal followed the order Fe>B>Cu>Mn>Zn. Fe was the most removed nutrient accounting for 121, 101 and 62 mg tree^{-1} for pear, apple and cherry, respectively (Figure 3). Removed micronutrients resulted always higher in pear compared to cherry trees and with the exception of Fe and Mn, also compared with apple (Figure 3). Apple trees removed higher amount of Mn, Cu and Zn than cherry, while comparable values were measured for Fe and B (Figure 3).

Table 3 reports, within each species, correlation parameters between total mineral removal and plant dry biomass. Pooling data of the 3 species, a linear significant (P<0.0001) correlation between total N and plant dry biomass was observed, with an R^2=0.97 (Figure 4).

Considering 1 ha nursery, despite the higher planting density of the cherry trees (+33% than apple and pear), the amount of removed nutrients mirrored that of individual tree (Figures 5 and 6). In fact, pear was the most nutrient demanding species in the nursery, then apple and cherry, either for macro and micronutrients (Figures 5 and 6). N was the most absorbed nutrient and pear trees removed slightly less than 200 kg N ha^{-1} while apple and cherry trees removed 107 and 100 kg N ha^{-1} (Figure 5), respectively. Similarly to N, high amounts of K and Ca were used by pear (~130-140 kg ha^{-1}) and apple trees (~50 and 130 kg ha^{-1} of K and Ca, respectively) while ~25 kg K ha^{-1} and 55 kg Ca ha^{-1}

Table. 1. Main chemical and physical characteristics of the soil of the nursery (average±standard error; n=3).

Parameter	Value
Coarse sand (g 100 g^{-1})	41.5±4.4
Fine sand (g 100 g^{-1})	36.5±4.4
Coarse silt (g 100 g^{-1})	5.5±1.8
Fine silt (g 100 g^{-1})	9.6±3.4
Lime (g 100 g^{-1})	6.9±6.1
Organic matter (g 100 g^{-1})	2.2±0.7
pH	7.6±0.2
Electrical conductivity (µS cm^{-1})	195±30
CEC (meq 100 g^{-1})	16.3±1.8
Total $CaCO_3$ (g 100 g^{-1})	7.9±2.4
Active lime ($CaCO_3$) (g 100 g^{-1})	3.2±1.0
N (g 100 g^{-1})	0.11±0.03
C/N	11.6±4.7
Organic C (g 100 g^{-1})	1.28±0.4
Available P (mg kg^{-1})	204±9.4
Exchangeable K (mg kg^{-1})	330±52
Exchangeable Mg (mg kg^{-1})	102±42

CEC, cation exchange capacity.

Table 2. Mineral concentration of roots, rootstock and variety of nursery cherry, apple and pear trees at the end of the growing phase.

	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Roots			(g DW kg^{-1})						(mg DW kg^{-1})		
Cherry	14.8^{a}	1.25	3.09^{c}	9.72^{ab}	1.05^{b}	0.66^{b}	1006^{a}	20.7^{ab}	10.5^{ab}	10.5^{b}	143.5^{a}
Apple	13.2^{b}	1.18	4.19^{b}	13.2^{a}	1.26^{a}	1.06^{a}	783^{ab}	33.0^{a}	10.0^{b}	20.3^{a}	118.1^{b}
Pear	11.9^{c}	1.40	6.14^{a}	8.27^{b}	1.28^{a}	0.55^{c}	472^{b}	15.1^{b}	14.4^{a}	8.60^{b}	115.7^{b}
P value	0.0004	0.0573	0.0001	0.024	0.0402	<0.0001	0.0302	0.0114	0.027	0.0002	0.0016
Rootstock			(g DW kg^{-1})						(mg DW kg^{-1})		
Cherry	7.82^{a}	0.59	1.45^{c}	4.68^{b}	0.45^{b}	0.37^{b}	208^{a}	8.60^{b}	7.63^{b}	7.68^{a}	122
Apple	6.81^{ab}	0.67	3.21^{b}	13.9^{a}	0.48^{b}	0.47^{a}	65.6^{b}	14.1^{a}	23.3^{a}	4.44^{b}	107
Pear	6.14^{b}	0.68	4.11^{a}	7.34^{b}	0.89^{a}	0.29^{c}	144^{a}	11.2^{ab}	17.7^{ab}	8.58^{a}	118
P value	0.0062	0.0594	<0.0001	<0.0001	<0.0001	<0.0001	0.0027	0.0243	0.0087	0.0012	0.144
Variety			(g DW kg^{-1})						(mg DW kg^{-1})		
Cherry	9.16	0.78^{b}	3.25^{b}	4.38	0.64^{b}	0.35^{b}	42.5	10.2^{a}	9.68^{b}	8.16	132
Apple	9.07	0.92^{ab}	3.55^{b}	5.39	0.70^{b}	0.47^{a}	29.7	12.3^{a}	34.9^{a}	9.16	123
Pear	8.84	0.98^{a}	4.70^{a}	4.00	1.04^{a}	0.39^{b}	43.1	5.08^{b}	29.4^{a}	10.7	123
P value	0.889	0.0375	0.0003	0.154	0.0008	0.0047	0.0746	0.0040	0.0003	0.0794	0.161

DW, dry weight. Root and rootstock tissues were 2-year old, while wood canopy was 1-year old. $^{a-c}$Within each organ, means followed by the same letter in the same column are not statistically different (P<0.05, Tukey's honest significant difference test).

were calculated for cherry trees (Figure 5). Removal of other nutrients was less pronounced and accounted for 22 and 29 kg ha^{-1} for P and Mg in pear trees, respectively, while for the same elements the amount did not exceed 10 kg ha^{-1} in the other species (Figure 5).

Among micronutrients, Fe was the most required (~3 kg ha^{-1}) independently of the species (Figure 6). B removal ranged between 1.2 and 2.4 kg ha^{-1} (80, 40 and 30 mg tree^{-1} for pear, apple and cherry, respectively) whereas Mn, Cu and Zn accounted for few hundred g ha^{-1}, irrespective of the species (Figure 6).

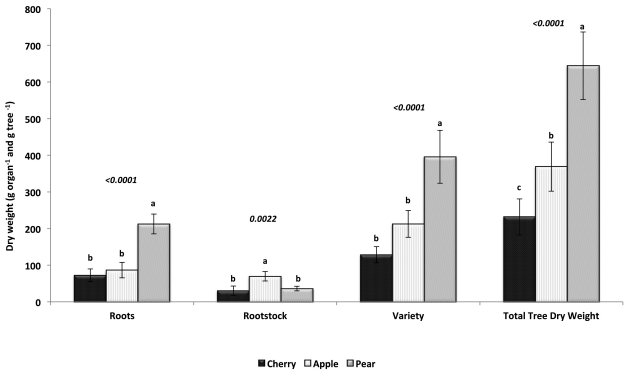

Figure 1. Organ dry biomass partitioning by nursery cherry, apple and pear trees. Roots and rootstock tissues were 2-year old while wood canopy was 1-year old. Within each organ, columns with different letters indicate statistical difference among species (P≤0.05 Tukey's test). Bars indicate confidence interval at 95%.

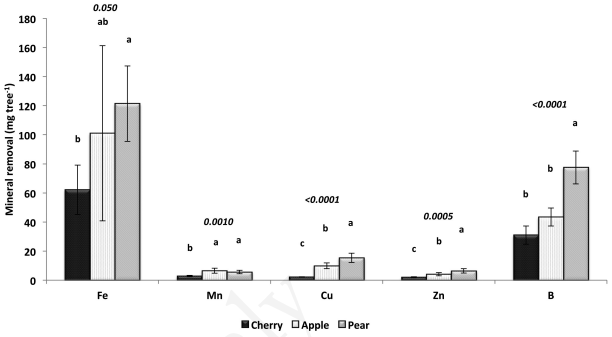

Figure 3. Micronutrients removed (mg tree^{-1}) by nursery cherry, apple and pear trees. Within each micronutrient, columns with different letters indicate statistical difference among species (P≤0.05 Tukey's test). Bars indicate confidence interval at 95%.

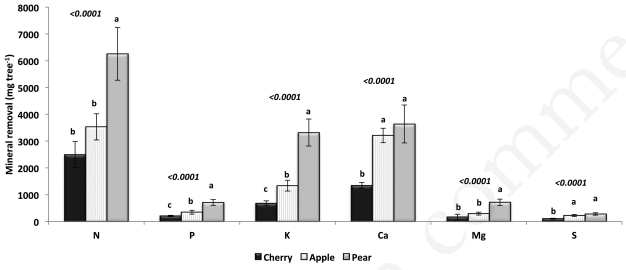

Figure 2. Macronutrients removed (mg tree^{-1}) by nursery cherry, apple and pear trees. Within each macronutrient, columns with different letters indicate statistical difference among species (P≤0.05 Tukey's test). Bars indicate confidence interval at 95%.

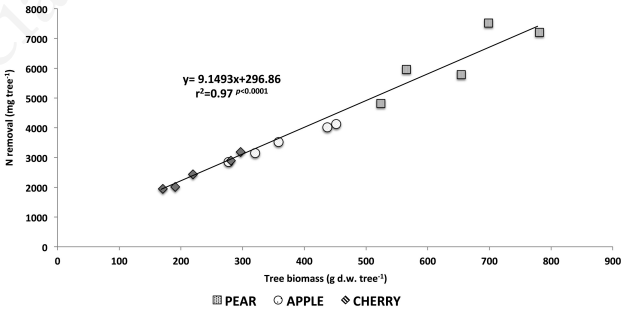

Figure 4. Correlation between tree dry biomass and nitrogen removed by nursery cherry, apple and pear trees. The line reports the trend of the three species.

Table 3. Pearson correlation between total plant dry biomass and total macro- and micronutrients removed by cherry, pear and apple nursery trees.

Element	Cherry			Pear			Apple		
	y	R^2	P values	y	R^2	P values	y	R^2	P values
Macronutrients									
N	9.7525x + 234.9	0.9812	0.0011	9.1703x + 346.44	0.7354	0.0631	7.2812x + 845.05	0.9856	0.0001
P	0.5252x + 84.299	0.8554	0.0244	1.0995x + 1.5699	0.8914	0.0157	1.0569x - 43.63	0.9389	0.0065
K	1.7701x + 268.69	0.9360	0.0070	5.2526x - 68.105	0.9099	0.0118	2.9086x + 263.61	0.9226	0.0094
Ca	-1.0133x + 1578.9	0.2239	0.4208	6.4798x - 538.76	0.7077	0.0741	3.8012x + 1806.8	0.878	0.0188
Mg	0.1507x + 132.3	0.5102	0.1753	1.1356x - 16.102	0.8075	0.0381	0.6468x + 53.73	0.6479	0.1003
S	0.3425x+23.965	0.9834	0.0009	0.4518x - 10.629	0.8065	0.0385	0.5863x + 8.7238	0.9704	0.022
Micronutrients									
B	0.127x + 1.568	0.9884	0.0005	0.1132x + 4.5605	0.8459	0.0270	0.0884x + 10.865	0.905	0.0128
Fe	-0.2983x + 131.3	0.7497	0.0578	0.1475x + 26.364	0.2741	0.3653	0.4222x - 54.674	0.2203	0.4251
Mn	-0.0082x + 4.7807	0.7022	0.0767	0.0095x - 0.4797	0.5559	0.1472	0.0177x - 0.0369	0.4827	0.1932
Cu	0.002x + 1.6915	0.2738	0.3582	0.0307x - 4.4244	0.7992	0.0407	0.0128x + 5.147	0.1858	0.4689
Zn	0.0062x + 0.5819	0.8744	0.0196	0.0145x - 2.9263	0.7637	0.0525	0.0159x - 1.772	0.9223	0.0095

Discussion

Genetic source and tree architecture significantly affected tree biomass partitioning. These differences were driven, other than by the vegetative behaviour, the age of the rootstocks (2 and 3-year old for cherry and pome trees, respectively) or the grafting compatibility, more likely by the reiterate application of benzyladenine mixed with gibberellins (*i.e*. GA4 and GA7) that were applied only to pome fruit trees with the aim to promote lateral shoot formation, as usually reported for these species (Palmer *et al.,* 2011). The interactions of such factors mirrored the biomass partitioning. Santa Maria, the pear variety adopted in our study, is described as highly vigorous cultivar with a good compatibility when grafted onto quince (Ikinci *et al.,* 2014) while apple G. Delicious and the Hungarian cherry Alex^{TM} are of medium (Barritt *et al.,* 1996) and low (Bassi, 2010) vigour, respectively. Despite the same age, roots of the dwarfing quince Adams (widely used as a rootstock for pear) showed a significantly higher biomass development compared to EMLA 9 (+145%) and, as expected, compared to the 1-year younger Gisela® 6 (+154%). This indicates that the pear variety was sustained by a well-developed root system while the vigour of apple was most likely reduced by the adopted rootstocks. Although dwarfing rootstocks are worldwide adopted to control tree size in intensive commercial orchards (Gregory *et al.,* 2013), it is worth to mention that within each species, different grafting combinations, in terms of genetic materials, will likely affect the results we observed in this study, thereby estimations should be adapted to the peculiar conditions.

Given that concentration among species resulted within the same order of magnitude, differences observed in tree nutrient removal were mainly driven by the tree biomass, as showed by the relationship between nutrient removal and tree biomass, explaining most of the observed responses.

Consequently, pome fruit trees resulted more nutrient demanding than cherry, despite the higher density of the latest (40,000 *vs* 30,000 trees ha^{-1}). Despite trees were non-bearing and characterised by a limited dry biomass, the estimated amount of minerals removed by 1 ha nurseries is higher compared to bearing trees of the same species grown in commercial orchards located in the same area (Sorrenti, 2006; Sorrenti and Rombolà, 2006; Toselli *et al.,* 2006; Tagliavini and Quartieri, 2008). Our findings are then in agreement with results proposed by Neto *et al.* (2008), who estimated that N requirements of young pear cv. Rocha grafted on quince BA29 trees were 3, 5 and 14 g N tree^{-1} over the first 3 years after planting. The same authors found that N requirements increased exponentially with tree age and were mainly correlated with the increase of trunk biomass (which represented the main N storage organ).

The high rate of nutrients removed in our experiment is a consequence of the ultra-high density adopted in nurseries and to the fact that trees are fully removed at the end of each growth cycle. However, while the amount of nutrients removed by the wood canopy coincide with the mineral uptake of the current season, the amount we found for rootstocks (including roots) represents the cumulative amount absorbed in 2 and 3 seasons for cherry and pome fruit species, respectively.

Nevertheless, for Gisela® 6, the amount of nutrients removed before transferring the acclimated plantlets in the open-field nursery is negligible compared to the subsequent uptake. Similarly, the amount of nutrients removed by rooted lignified shoots (M9 and quince Adams) generated in the mound layering was probably scarce. However, as Gisela 6® rootstocks are grafted in the same season of their production while pome fruit species are 1-year older (rootstocks grown 1 year in the mound layering), the amount of nutrients absorbed by the entire rootstock (including roots) of a *ready-to-plant* tree is a consequence of two consecutive growing seasons in the nursery, irrespective of the species.

So that, the nutrient requirements we calculated per tree may appear overestimated, as part of the nutrients we measured were immobilised in the perennial structure of the rootstock in the previous growing seasons. Nevertheless, as the 1-year old canopy mostly contributed (>50%) to the total tree dry biomass, the mineral absorption of the ungrafted rootstocks (the growing season in the nursery previous grafting in the case of pome fruit species), should be less than 25% of the total amount.

On the other hand, we underline that the rate of nutrients consumed by the biomass of the removed shoots (aerial part of the rootstock after grafting, as well as that of the suckers) and by leaves was not considered in this study. However, leaves and shoots remain in the nursery, as the latter are mechanically chipped and incorporated into the soil. Nevertheless, although such fractions do not represent a net loss from the agroecosystem as the minerals return to the soil after mineralisation (Tagliavini *et al.,* 2007), absorbed rates should be considered in the fertilisation schedules.

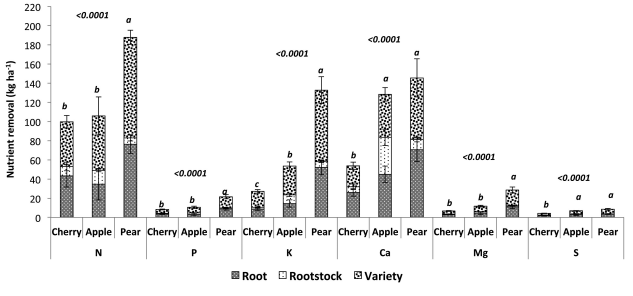

Figure 5. Macronutrients removed (kg ha^{-1}) by nursery cherry, apple and pear tree organs. Root and rootstock tissues were 2-year old, while wood canopy was 1-year old. Within each mineral, columns with different letters indicate statistical difference among the species total removal (P≤0.05 Tukey's test). Bars indicate confidence interval at 95%.

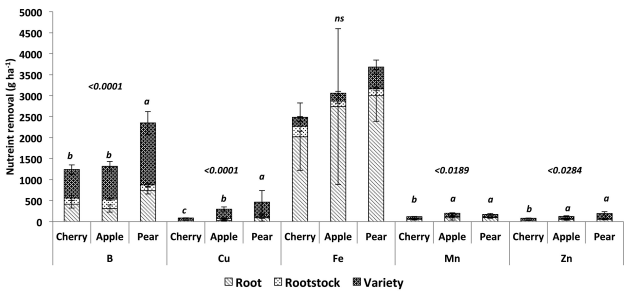

Figure 6. Micronutrients removed (g ha^{-1}) by nursery cherry, apple and pear tree organs. Root and rootstock tissues were 2-year old, while wood canopy was 1-year old. Within each mineral, columns with different letters indicate statistical difference among the species total removal (P≤0.05 Tukey's test). Bars indicate confidence interval at 95%.

N was the most removed element by all the 3 grafting combinations, as it represents the most important nutrient for young non-bearing trees. Although N must be not limiting in the nursery growing substrate, it requires accurate management as its mineral forms (*i.e.* NO_3-N) in soil are scarcely retained by colloids, especially in sandy and poorly structured soils (as often preferred for nurseries), with risks of groundwater potential pollution.

Based on these evidences, we recommend to split the total N rate in several applications (*e.g.* by fertigation) to assure adequate mineral N availability to plants without excess, increasing thereby the N use efficiency and avoiding detrimental economic and ecological effects.

The relatively high amount of Fe absorbed by plants confirms that Fe nutrition must be adequately managed particularly in alkaline-calcareous soils, which are prone to limit Fe availability for plants (Sorrenti *et al*., 2011). In such conditions, the supply of organic amendments before the establishment of the nursery plots or the adoption of synthetic Fe-chelates fertilisers may result effective in managing Fe-nutrition of young trees.

Considering the limited amount required, the supply of micronutrients in the nursery does not seem to pose particular concerns as it is provided through the reiterate use of foliar-applied pesticides, often based on Zn, Mn and especially Cu or S. It is worth mentioning that our estimates are referred to the specific site, density and climate conditions of the nursery, thereby fertilisation strategies in other conditions should be adapted.

Conclusions

Although in this study pome fruit species showed higher nutrient removal compared to cherry trees, we believe that this does not indicate that they are more nutrient demanding compared to stone fruit species, rather the amount of nutrient required is a matter of plant vigor. The amount of nutrient uptaken by trees was directly related to the tree growth and dry biomass, thus depending on the grafting combination, soil fertility, climate, *etc*.

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