Nutr Cycl Agroecosyst (2016) 105:39–50
DOI 10.1007/s10705-016-9772-3

ORIGINAL ARTICLE

Organic fertilization in nectarine (*Prunus persica* var. *nucipersica*) orchard combines nutrient management and pollution impact

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Received: 14 December 2015 / Accepted: 15 March 2016 / Published online: 21 March 2016

Abstract Recycled organic fertilizers may be used to replace chemical source of nutrients; however, some nutrients such as nitrogen and heavy metals released by mineralization can become potential pollutants. The objective of this experiment was to compare, over a 3-year-period of time, the effectiveness of two organic fertilizers (cow manure and compost) with a traditional mineral fertilizer on soil fertility, tree nutritional status, heavy metal concentration in soil and plant in a mature nectarine orchard. Trees were subjected, since their plantation (made in 2001) to the following treatments: (1) mineral fertilization (including nitrogen at 130 kg ha^{-1} $year^{-1}$); (2) cow manure (5 t DW $year^{-1}$ ha^{-1}); (3) compost (5 t DW $year^{-1}$ ha^{-1}). Soil organic matter and total nitrogen concentration increased as a consequence of compost application. Soil nitrate concentration was increased by mineral fertilizer and compost applications. In summer, macro and micro nutrient concentrations in leaves were not affected by treatments with the exception of N that was increased by mineral fertilization. At the end of the season, leaf N, K and Zn were remobilized to storage organs, while Ca, Mg, Cu, Fe and Mn accumulated in abscised leaves and returned to the soil, with no differences among treatments. Nitrogen and K were found principally in fruit flesh. With the exception of Cu and Zn, the concentration of heavy metals in leaves and fruits was below detection limits. Total and DTPA-extractable heavy metals in soil were not increased by organic fertilization if compared with mineral fertilizer.

Keywords Compost · Cow manure · Nutrient remobilization · Nitrogen · Heavy metals

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Introduction

The doubling of agricultural food production during the past 40 years has been associated with an increase in crop fertilization. Over application of chemical fertilizers in intensive agricultural systems has lead to excessive accumulation of nutrients in groundwater (Volk et al. 2009). It has been estimated that 55 % of water pollution in the EU is caused by agriculture (Kersebaum et al. 2003), since losses of N and P are responsible for water quality impairment in lakes and rivers (Potter et al. 2006). Less than 50 % of the amount of mineral N applied with fertilizations is taken up by woody plants, depending on fertilizer efficiency, plant and soil type, climatic conditions and agricultural practices (Carranca 2012). The detrimental impact of agriculture on the environment can be reduced by an appropriate management of fertilization aimed to minimize nutrient losses. A valuable alternative to mineral fertilization could be the application of organic fertilizers such as cow manure or compost that, recently, has showed a positive effect on soil properties (Diacono and Montemurro 2010; Baldi et al. 2010), yield and plant nutritional status (Gallardo-Lara and Nogales 1987; Baldi et al. 2014). The availability and quality of municipal solid waste (MSW) composts have increased and compost now represents a source of high quality organic matter (OM) that, beside the fertilization value (Gallardo-Lara and Nogales 1987), contributes to recycle municipal solid and food-industry-related-wastes.

The main issue related to organic fertilizer application is the synchronization of nutrient release with plant needs; applying nutrients in accordance with plant requirements is crucial to maximize nutrient efficiency and maintain plant productivity (Neilsen et al. 2001; Aguirre et al. 2001). Another concern regarding the safe use of organic fertilizers in agriculture is the possible presence of heavy metals that have a negative impact on the environment. Heavy metals can accumulate in agricultural soils, leach into soil solution and reach the groundwater and, in the long term, they may contaminate both human and animal food chain (Nawab et al. 2015; Rahman et al. 2014; Singh and Kalamdhad 2013).

The aim of the present study was to compare the effectiveness of two organic fertilizers, such as traditional cow manure and more recently introduced MSW compost, with a traditional mineral fertilizer in nectarine trees nutrient management, to verify their effect on soil fertility, tree nutritional status, heavy metal concentration in soil and plant.

Methods

Plant material and treatments

The study was carried out from 2010 to 2014, at the experimental farm "M. Marani" near Ravenna, Italy, in the south-eastern part of the Po valley (44°27′N; 12°13′ E). The key characteristics of the Calcaric Cambisol (FAO 2010) soil of the orchard are summarized in Table 1. The area is characterized by a temperate climate where the annual precipitation ranges between 500 and 750 mm and the average temperature is 13.7 °C (1967–1997; Geophysical Station of Modena University). The trial was conducted on a nectarine [*Prunus persica*, Batsch var. *nucipersica* (Bockh.) Schn.] orchard of the variety Stark RedGold, grafted on hybrid GF677 (*Prunus persica* × *Prunus amygdalus*). The trees, trained as in a "delayed-vasette" system, were planted on August 2001 at the distance of 5 m between rows and 3.8 m between trees along the row. The orchard was regularly watered from June to September with a drip irrigation system to return the daily evapotranspiration rate, measured by the local meteorological station. The soil was tilled superficially (0.25 m) in a 2 m wide tree row, while alleys were covered with spontaneous grass mowed 3 times a year.

Table 1 Soil physical and chemical characteristics at the beginning of the trial (average of 4 replicates ± standard deviation)

Soil characteristics	
Sand (g kg^{-1})	67.0 (±1.5)
Silt (g kg^{-1})	670 (±1.41)
Clay (g kg^{-1})	262 (±1.71)
pH	7.8 (±0.05)
Ca carbonate (g kg^{-1})	305 (±1.29)
Active lime (g kg^{-1})	125 (±1.29)
Organic matter (g kg^{-1})	16.8 (±0.13)
K_2O extractable (mg kg^{-1} DW)	219.5 (±33.7)
P_2O_5 Olsen (mg kg^{-1} DW)	42.5 (±2.38)
Cd (mg kg^{-1} DW)	<0.5
Cr (mg kg^{-1} DW)	35.5 ± 1.73
Cu (mg kg^{-1} DW)	24.7 ± 0.50
Hg (mg kg^{-1} DW)	0.18 ± 0.04
Ni (mg kg^{-1} DW)	64.2 ± 1.50
Pb (mg kg^{-1} DW)	33.5 ± 0.58
Zn (mg kg^{-1} DW)	73.7 ± 0.96
C.E.C. (meq 100 g^{-1} DW)	10.1 (±1.95)
Electrical conductivity (μS cm^{-1})	200 (±8.2)

Since orchard plantation, the following treatments were compared, as in a complete randomised block design, with four replicates: (1) mineral fertilization, according to Integrated Crop Management Guideline of the region Emilia-Romagna (www.RegioneEmilia-Romagna.it), that included P, at 100 kg ha^{-1}, and K, at 200 kg ha^{-1}, applied only at planting and N (70 kg ha^{-1} $year^{-1}$), split in May (60 % of the total dose) and September (40 %). In 2004, N application rate was increased to 120 kg ha^{-1} year^{-1}, and from 2006 to 130 kg ha^{-1} year^{-1}; (2) cow manure, applied at planting at 10 t dry weight (DW) ha^{-1} and from 2004, in a single application of 5 t ha^{-1} in May corresponding to an application of 80 kg N ha^{-1} year^{-1}; (3) compost, applied at planting at 10 t DW ha^{-1} and, from 2004, applied in May in a single application of 5 t ha^{-1} equal to 120 kg N ha^{-1} year^{-1}. Each replicate plot included six trees, only the four central were used for data collection. Organic fertilizers were applied on 2 m-wide-strip, on tree row for a surface of 4000 m^2 ha^{-1} and tilled into the soil at 0.25 m of depth. According to local farm management, pruned wood was chopped and left on the ground of the orchard.

Amendment composition

Cow manure was obtained by cow stable dung and wheat straw bedding after a 6-month-stabilization and provided by a local cow livestock (Table 2). Compost was obtained from municipal solid wastes (50 %) mixed with pruning material from urban ornamental trees and garden management (50 %) after 3-month stabilization (Table 2). Compost and cow manure were analysed by and external public laboratory (ARPA, Ravenna, Italy). In particular, organic matter was determined according to the Springer–Klee method that is based on oxidation of OM by $K_2Cr_2O_7$ in the presence of H_2SO_4 at 160 °C. The amount of organic C was calculated by back titration of the dichromate with a $FeSO_4$ solution in the presence of a ferroine indicator. Total N was determined using an elemental analyser. A sample of amendment was extracted with NaOH and $Na_4P_2O_7$; humic carbon (humic+ and fulvic fraction) was separated by an oxidative digestion with K_2Cr2O_7 and H_2SO_4 and determined through titration with $FeSO_4$ as described previously for OM.

Table 2 Selected chemical characteristics of compost and cow manure used in the trial

Parameter	Compost	Cow manure
pH	8.75	9.15
DW (g kg^{-1})	821	327
N (g kg^{-1} DW)	24.0	16.3
N–NH_4 (mg kg^{-1} FW)	843	1745
TOC (g kg^{-1} DW)	228	272
C/N	10	17
P (g kg^{-1} DW)	58	59
K tot. (g kg^{-1} DW)	91	234
EC (mS m^{-1})	412	263
HA + FA (g kg^{-1} DW)	79.2	121
HI	0.62	0.36
Cu (mg kg^{-1} DW)	122	–
Zn (mg kg^{-1} DW)	365	–
Pb (mg kg^{-1} DW)	100	–
Ni (mg kg^{-1} DW)	41	–
Cd (mg kg^{-1} DW)	0.59	–
Hg (mg kg^{-1} DW)	0.15	–
Cr VI (mg kg^{-1} DW)	0.25	–

DW dry weight, *NH_4^+-N* ammonium N, *TOC* total organic carbon, *EC* electric conductibility, *HA + FA* humic acid + fulvic acid, *HI* humification index (ratio between non humified C:humified C)

Soil analysis

Every year in September, soil samples were collected at the depth of 0.03–0.40 m to evaluate, by an external public laboratory (ARPA, Ravenna, Italy), soil OM and total N according to the procedure of the Italian Ministry of Agriculture, Food and Forestry and the International Union of Soil Sciences (Violante 2000) and as described previously for compost. To assess the effect of treatments on soil nitrate (NO_3^-) and ammonium (NH_4^+)-N, soil cores were collected at a depth of 0–0.40 m three times per year (spring, summer and autumn). Nitrate- and ammonium-N were extracted from 10 g of soil by a solution of 100 mL of KCl (2 mol L^{-1}); samples were shaken at 110 rpm for 1 h and, after soil sedimentation, limpid solution was collected and stored at −20 °C until analysis made with an auto analyzer (Auto Analyzer AA-3; Bran + Luebbe, Norderstadt, Germany). In summer 2014, soil samples were collected at the depth of 0.15 m to measure the concentration of heavy metals; soil was then sieved at 2 mm and oven-dried before analysis of total and extractable heavy metals. Total soil heavy metals were determined by wet mineralization by treating 0.5 g of dry soil with 6 mL of HCl (370 mL L^{-1}), 2 mL of HNO_3(650 mL L^{-1}) and 2 mL of H_2O_2 (300 mL L^{-1}) in an Ethos TC microwave labstation (Milestone, Bergamo, Italy). Solution were filtered with filter papers (Whatman 42®) and the concentration of Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn was determined by plasma spectrometer

(Ametek Spectro, Arcos, Kleve, Germany). To evaluate the amount supplied with fertilizers, the fraction of DTPA-extractable heavy metals was determined according to Lindsay and Norvell (1978) modified as follows: 5 g dry soil were shaken for 2 h at 60 cycles per min with 25 mL of a solution made with DTPA 1.97 g L^{-1}, triethanolamine 14.92 g L^{-1} and $CaCl_2$ 1.46 g L^{-1} buffered to pH 7.3 with HCl (Leita and Petruzzelli 2000). The suspension was centrifuged at 3000 rpm for 5 min; supernatant was collected, filtered with filter papers (Whatman 42®) and analysed as described before.

Plant analysis

Every year in July, a sample of the 20 youngest, fully expanded leaves was collected from the apical part of shoots; leaf chlorophyll was measured by a portable SPAD 502 (Minolta Co., Ramsey, NJ, USA). Leaves were then washed, oven-dried, weighed, milled and analyzed for N, P, K, Ca, Mg, Cu, Fe, Mn and Zn concentration. Concentration of N was evaluated by C/N elemental analyser (Carlo Erba, Milan, Italy) coupled with a mass spectrometer (Delta plus Finnigan, Bremen, Germany). Metal concentrations were determined by atomic absorption spectrophotometer (SpectrAA-200, Varian, Mulgrave, Victoria, Australia) on a 0.3 g sample previously mineralized (US EPA Methods 3052; Kingston 1988) in an Etos TC microwave lab station (Milestone, Bergamo, Italy). Phosphorus was spectrophotometrically quantified at 700 nm, through extract mineralization (Saunders and Williams 1955) of 0.5 g of the sample with H_2SO_4 (960 mL L^{-1}) and H_2O_2 (35 mL L^{-1}), and subsequent neutralization with 0.1 M NaOH enriched with 0.1 mol L^{-1} ascorbic acid, 32 mmol L^{-1} ammonium molybdate, 2.5 mol L^{-1} H_2SO_4 and 3 mmol L^{-1} potassium antimonyl tartrate to develop a phosphomolybdic blue colour. In July 2011, leaf area was also measured (Portable area meter Li-3000, LiCor inc., Lincoln, Nebraska), and specific leaf weight (SLW) was calculated by dividing the DW of the 20 leaf-samples without petioles by their area. In 2011, leaf nutrient concentration was also reported to SLW and expressed as g m^{-2} (for macronutrients) or mg m^{-2} (for micronutrients). Fruit thinning was carried out manually in May 2011 and DW was recorded. A sample of fruits was then dried, weighted, milled and analysed as previously described. In 2011 fruits at harvest were divided into flesh and stone and their fresh and DW was measured. Dried flesh and stone were milled, weighted and analysed as described for leaves. In September 2011, one tree per block was enclosed into a plastic net, to collect leaves naturally abscised at the end of the season. Periodically abscised leaves were picked and weighted and, on a representative sample of 100 leaves, leaf area was measured; leaves were then oven dried, weighted to calculate their SLW and analysed as previously described. Pruned wood (1 year old twigs and older branches) biomass was recorded in February 2012. On a sample properly dried and milled, macro and microelements were determined as described before. Concentration of heavy metals in leaves and fruit flesh, sampled in 2011, was determined by plasma spectrometer (Ametek Spectro, Arcos, Kleve, Germany) on the same samples digested for atomic absorption analysis. Total amount of macro and micro-nutrients was evaluated by multiplying their concentration in the organ (leaf, thinned fruits, pruned wood, fruit flesh and stone) by the organ DW.

Statistical analysis

Data of soil OM and total N, leaf chlorophyll and macronutrient concentrations, tree yield and fruit FW were statistically analyzed as in a factorial experimental design with soil fertilization (3 levels: mineral, cow manure and compost), and year (3 levels: 2010, 2011, 2012) as main factors.

Data of leaf mineral concentrations, collected in 2011 and reported on SLW, were statistically analyzed as in a factorial experimental design with soil fertilization (3 levels: mineral, cow manure and compost), and time (2 levels: July and December) as main factors. Data of nutrient accumulation within tree organs were statistically analyzed as in a factorial experimental design with soil fertilization (3 levels: mineral, cow manure and compost), and organ (5 levels: thinned fruits, fruit flesh, fruit stone, abscised leaves and pruned wood) as main factors. Heavy metal concentration in soil, leaves and fruits was analyzed according to a complete randomized block design. When analysis of variance showed a statistical effect of treatments ($P \leq 0.05$), means were separated by Student–Newman–Keuls test.

Concentration of Cu and Zn in soil (total and DTPA), leaves and fruits was also analysed according to a discriminant canonical analysis (DCA).

Results

Although no interaction between fertilization treatment and year was observed for soil OM and total N, Fig. 1 shows the effect of each treatment separated for each year. Soil OM was increased by application of compost, compared to cow manure and mineral fertilizer in 2011 and 2012; while in 2010 the positive effect was significant only compared to mineral fertilization (Fig. 1A). Total N was not influenced by

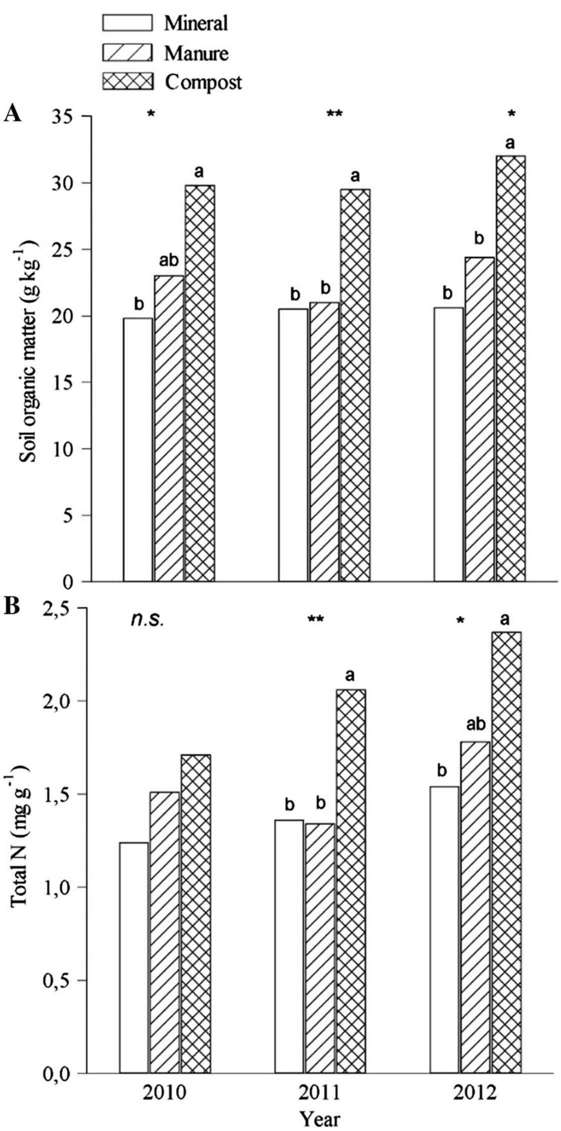

Fig. 1 Effect of fertilization treatment on soil organic matter (**A**) and total N (**B**) in the soil layer between 0.03 and 0.40 m of depth, from 2010 to 2012

fertilization treatment in 2010, in 2011 it was increased by application of compost, compared to cow manure and mineral fertilizer, while in 2012, total N was enhanced by compost only if compared to mineral fertilization (Fig. 1B).

Soil NO_3^--N concentration was increased in May and August 2010 by mineral fertilizer and in November 2011 and August 2012 by compost application (Table 3); no effect of treatment was observed in the other sampling dates. Soil NH_4^+-N concentration was not influenced by the fertilization treatments with the exception of data collected in November 2011 when it was increased by cow manure applications (Table 3).

Soil heavy metal total concentration was not influenced by fertilization treatment with the exception of Pb that was increased by compost amendment if compared with cow manure (Table 4). Soil DTPA-extractable heavy metal concentration was not affected by treatment; Co, Cr and Hg were below the detection limits (Table 4).

Fertilization treatment and year did not interact significantly with leaf chlorophyll and macronutrient concentration, consequently on Table 3 only the effect of main factors are presented. Leaf chlorophyll and N concentration were increased by mineral fertilizer. The other leaf macro (Table 5) and micro (data not tabulated) nutrients were not affected by treatments. On average of the 3 treatments, leaf chlorophyll decreased from 2010 to 2012; while leaf N concentration decreased from 2010 to 2011 and then in 2012 remained stable (Table 5). Leaf concentration of P was higher in 2012 than in 2010 and 2011, while the concentration of K, Ca and Mg were higher in 2011 than in the other 2 years (Table 5).

The concentrations of Cu and Zn in leaves and fruits were not affected by fertilization treatments (Table 6); Cd, Co, Cr, Hg and Pb leaf and fruit concentrations were below the detection limits (Table 6).

In summer and winter 2011, leaf nutrient concentrations (expressed on SLW) were not influenced by treatments (data not showed) so that only the effect of the time of sampling is reported (Table 7). At leaf abscission (December), concentration of N, K and Zn was lower, while concentration of Ca, Mg, Cu, Fe and Mn were higher than in July (Table 7); only leaf P concentration did not change from July to December (Table 7).

Fertilization and organ did not interact significantly with DW and nutrient accumulation, consequently in

Table 3 Effect of fertilization treatment on soil nitric (NO_3^-)-N and ammonium (NH_4^+)-N-concentration

Fertilizer	2010			2011			2012		
	May	August	November	May	August	November	May	August	November
NO_3^--N (mg kg^{-1} DW)									
Mineral	11.8 a	19.3 a	5.7	4.66	5.94	0.340 b	6.94	7.86 b	2.91
Manure	5.3 b	6.3 b	5.7	6.56	5.83	0.341 b	5.45	8.50 b	2.46
Compost	6.5 b	7.2 b	5.8	5.76	5.14	0.567 a	6.94	18.1 a	7.75
Significance	*	**	n.s.	n.s.	n.s.	**	n.s.	*	n.s.
NH_4^+-N (mg kg^{-1} DW)									
Mineral	5.7	0	0	6.94	5.45	4.00 b	3.75	2.71	3.40
Manure	7.1	0	0	4.90	5.36	6.70 a	4.56	2.36	5.38
Compost	6.7	0	0	5.86	5.70	4.96 b	5.79	3.60	5.04
Significance	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.

Values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$)

n.s., *, ** effect not significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Table 4 Effect of fertilization treatment on total and DTPA-extractable heavy metals

Fertilizer	Cd (mg kg^{-1})	Co (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Hg (mg kg^{-1})	Ni (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
Total								
Mineral	0.304	11.7	60.8	40.6	n.d.	47.32	17.9 ab	77.5
Cow manure	0.209	11.1	57.5	39.5	n.d.	46.05	16.7 b	73.7
Compost	0.219	11.8	63.4	44.4	n.d.	47.19	19.7 a	81.3
Significance	n.s.	n.s.	n.s.	n.s.	–	n.s.	*	n.s.
DTPA-extractable								
Mineral	0.039	n.d.	n.d.	5.71	n.d.	0.744	0.77	1.97
Cow manure	0.038	n.d.	n.d.	6.49	n.d.	0.715	0.70	2.38
Compost	0.043	n.d.	n.d.	5.87	n.d.	0.739	0.91	3.10
Significance	n.s.	–	–	n.s.	–	n.s.	n.s.	n.s.

Values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$)

n.d. not detected. Detection limit (mg kg^{-1}) for Co 0.002, Cr 0.002 and Hg 0.004

n.s., * effect not significant, significant at $P \leq 0.05$, respectively

Table 8 only the effect of main factors is reported. While not affected by treatments (Table 8), organ DW was higher in fruit flesh, followed by pruned wood, abscised leaves and fruit stones and finally thinned fruits. On average of all organs analysed, total amount of nutrients was not affected by treatments, with the exception of Cu and Fe that were increased by mineral fertilization. Nitrogen was found in higher amount in fruit flesh, abscised leaves and pruned wood than fruit stone and thinned fruits (Table 8). The amount of P was higher in abscised leaves and fruit flesh than in pruned wood, while the lowest amount was found in thinned fruits and fruit stones (Table 8). The amount of K was the highest in fruit flesh, followed by abscised leaves, thinned fruits, pruned wood, and was the lowest in fruit stones (Table 8). Calcium was mainly accumulated in abscised leaves, followed by pruned wood and only a small amount was found in fruits at harvest (flesh and stone) and at thinning (Table 8). The highest amount of Mg was found in abscised leaves, followed by pruned wood and fruit flesh, that were higher than fruit stones and thinned

Table 5 Effect of fertilization treatment and sampling year on chlorophyll (chl) and macronutrient leaf concentration

	chl (spad unit)	N (g kg^{-1} ss)	P (g kg^{-1} ss)	K (g kg^{-1} ss)	Ca (g kg^{-1} % ss)	Mg (g kg^{-1} ss)
Fertilizer						
Mineral	40.6 a	29.8 a	5.02	20.3	23.4	4.99
Cow manure	39.3 b	27.1 b	5.22	21.0	22.8	5.00
Compost	39.6 b	27.6 b	5.82	20.1	22.9	5.13
Significance	**	**	n.s.	n.s.	n.s.	n.s.
Year						
2010	41.0 a	31.0 a	4.66 b	18.5 b	22.8 b	3.81 c
2011	39.7 b	26.6 b	3.28 b	25.4 a	256 a	6.49 a
2012	38.8 c	26.8 b	8.11 a	17.5 b	20.8 c	4.82 b
Significance	*	**	***	***	***	***

Values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$)

n.s., *, **, *** effect not significant, significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. Interaction treatment × year not significant

Table 6 Effect of fertilization treatment on heavy metals concentration in leaves and fruits in 2011

Fertilizer	Cd (mg kg^{-1})	Co (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Hg (mg kg^{-1})	Ni (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
Leaves								
Mineral	n.d.	n.d.	n.d.	7.25	n.d.	n.d.	n.d.	22.7
Cow manure	n.d.	n.d.	n.d.	7.25	n.d.	n.d.	n.d.	23.1
Compost	n.d.	n.d.	n.d.	7.69	n.d.	n.d.	n.d.	24.6
Significance	–	–	–	n.s.	–	–	–	–
Fruits								
Mineral	n.d.	n.d.	n.d.	5.83	n.d.	n.d.	n.d.	7.74
Cow manure	n.d.	n.d.	n.d.	6.14	n.d.	n.d.	n.d.	8.06
Compost	n.d.	n.d.	n.d.	6.12	n.d.	n.d.	n.d.	8.52
Significance	–	–	–	n.s.	–	–	–	n.s.

n.s. effect not significant

n.d. not detected. Detection limit (mg kg^{-1}) for Cd 0.0003; Co 0.002; Cr 0.002; Hg 0.004; Ni 0.002 and Pb 0.006

Table 7 Effect of sampling date on specific leaf weight (SLW), macro and micro nutrient concentration in leaves

Sampling time	SLW (mg DW m^{-2})	N (mg DW m^{-2})	P (mg DW m^{-2})	K (mg DW m^{-2})	Ca (mg DW m^{-2})	Mg (mg DW m^{-2})	Cu (μg DW m^{-2})	Fe (μg DW m^{-2})	Mn (μg DW m^{-2})	Zn (μg DW m^{-2})
July	71.6	1.91	0.58	1.25	0.79	0.35	0.36	5.04	0.69	3.71
December	71.4	0.92	0.68	1.16	1.25	0.49	0.49	18.1	2.91	2.58
Significance	n.s.	***	n.s.	*	**	***	***	***	***	*

n.s., *;**, *** effect not significant, significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

Table 8 Effect of fertilization treatment and sampling organ on biomass dry weight (DW) and nutrient accumulation within nectarine tree organs in 2012

Fertilizer	Biomass (g DW $plant^{-1}$)	N (g $plant^{-1}$)	P (g $plant^{-1}$)	K (g $plant^{-1}$)	Ca (g $plant^{-1}$)	Mg (g $plant^{-1}$)	Cu (mg $plant^{-1}$)	Fe (mg $plant^{-1}$)	Mn (mg $plant^{-1}$)	Zn (mg $plant^{-1}$)
Mineral	3846	34.0	15.8	38.0	12.3	6.46	20.2 a	368 a	34.8	161
Cow manure	3521	30.9	14.5	34.8	10.9	5.77	17.7 b	253 b	33.0	137
Compost	3378	32.2	13.2	33.9	10.1	5.75	17.9 b	235 b	35.0	154
Significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.
Organ	**Biomass (g DW $plant^{-1}$)**	**N (g $organ^{-1}$)**	**P (g $organ^{-1}$)**	**K (g $organ^{-1}$)**	**Ca (g $organ^{-1}$)**	**Mg (g $organ^{-1}$)**	**Cu (g $organ^{-1}$)**	**Fe (g $organ^{-1}$)**	**Mn (g $organ^{-1}$)**	**Zn (g $organ^{-1}$)**
Thinned fruits	1744 d	26.4 b	7.81 c	27.7 c	1.02 c	2.03 c	15.6 c	79.4 c	8.13 c	59.3 bc
Fruit flesh	6933 a	37.7 a	22.1 a	88.7 a	2.55 c	4.45 b	28.7 a	109 c	17.1 c	97.5 b
Fruit stone	2283 cd	25.0 b	3.09 c	3.00 e	0.731 c	1.22 c	8.08 d	129 c	8.54 c	38.6 c
Abscised leaves	2652 c	34.4 a	24.6 a	43.4 b	45.4 a	18.2 a	18.3 c	684 a	109 a	95.4 b
Pruned wood	4296 b	38.2 a	14.8 b	15.0 d	5.87 b	4.06 b	22.3 b	426 b	28.3 b	463 a
Significance	***	**	***	***	***	***	***	***	***	***

Values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$)

n.s., *, **, *** effect not significant, significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. Interaction treatment × organ not significant

fruits (Table 8). Copper amount was largest in fruit flesh, followed by pruned wood, abscised leaves and thinned fruits (not statistically different) and finally by fruit stones (Table 8). Iron and Mn accumulation was the highest in abscised leaves, followed by pruned wood and by the other organs (Table 8). Zinc was accumulated in highest amount in pruned wood followed by abscised leaves and fruit flesh, thinned fruits and fruit stones (Table 8).

According to the discriminant canonical analysis a net separation between concentration in soil (on the left of Fig. 2) and in vegetal organs (on the right of Fig. 2) was found. Gradient 1 explained 54 % of the variability and was negatively (−0.92) correlated with Zn concentration; gradient 2 explained the other 46 % of the variability and was negatively (−0.80) correlated with Cu concentration.

Discussions

The results of the present investigation showed that, in the long term, the annual application of compost (that started in 2001) almost doubled the concentration of soil OM (that increased from 16.5 to 30 g kg^{-1}). The effect of cow manure on the increase of soil OM and N was lower than compost and similar to mineral fertilizer. This response was probably the results of a faster rate of degradation of the OM applied by cow manure compared with compost, as a result of the different organic material and composting time of the two amendments. However, this different behaviour did not increase mineral N concentration in soil treated with cow manure meaning that losses of N from the orchard ecosystem different from NO_3^- leaching, such as gaseous N (Chadwick et al. 2011), occurred. Unexpectedly, mineral fertilization increased soil OM to a similar extent of cow manure, this was a possible consequence of soil management that included a 3-m central alley covered with spontaneous grass, that was mowed three times per year and, along with the root turnover, contributed to increase soil OM.

The oxidation of ammonium to nitrate is controlled by the availability of N, soil temperature, soil moisture, soil texture and organic carbon. According to Burger and Jackson (2003) the application of OM

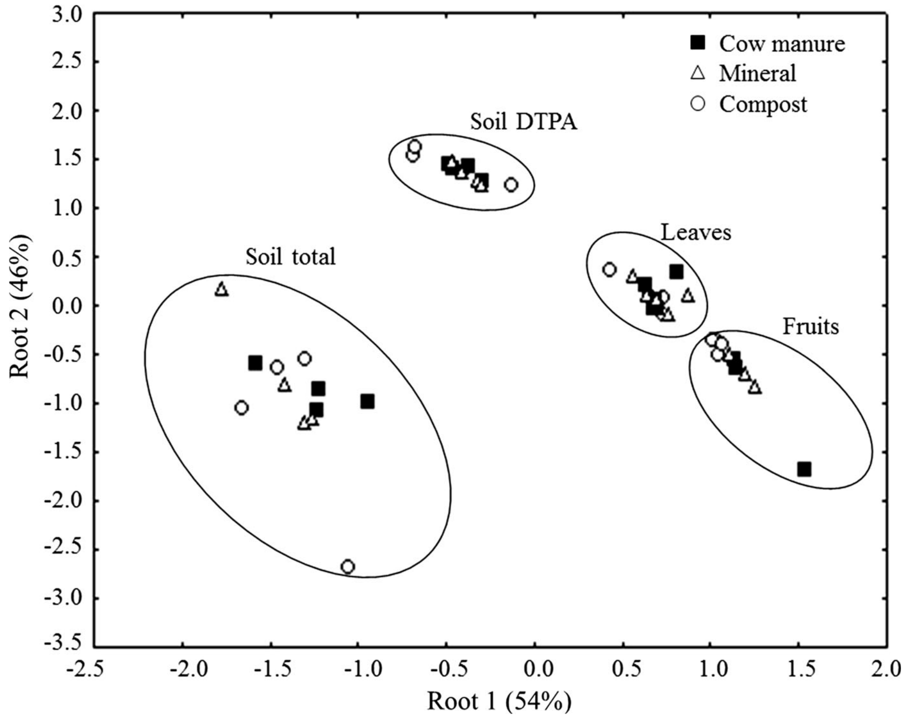

Fig. 2 Discriminant canonical analysis on Cu and Zn concentration in soil (total and DTPA-extractable), leaves and fruits

resulted in a slower rate of release of inorganic N than mineral fertilization as also testified by the application of mineral fertilizer that enhanced the nitrate–N availability in 2010 compared to the other treatments with a consequent increase, in this season, of leaf N concentration.

In our study no effect on tree yield was observed, showing that the different soil mineral N induced by treatments was not sufficient to modify tree yield. Overall, soil mineral N concentration observed in 2010 and 2012 were in the lower range (5–20 mg kg^{-1}) established as optimal for peach growth and production (Tagliavini et al. 1996), this contributed to reduce the risk of environment contamination, but at the same time may have limited the nectarine tree performance in term of shoot and fruit growth. Nevertheless, leaf analysis made in July, showed a concentration of macro and micronutrients within the optimal range for Stark RedGold in Emilia-Romagna Region (Baldi et al. 2004). Nutrient concentrations found in tree organs were lower than those found in a similar study in Spain (El-Jendoubi et al. 2013), with the exception of N in fruit stones, P in fruits (flesh and stone), and Zn in fruit flesh and pruned wood that, in this experiment, were higher than those observed in Spain (El-Jendoubi et al. 2013). The discrepancy could be related to variety, orchard management, crop load, environment and soil properties. However, the pattern of nutrient allocation into organs were similar in the two experiments. Fruit was the organ that most of all contributed to nutrient removal, among them, K was the most abundant with Ca and Mg the least.

In 2011, the decrease of the concentration of N, K and Zn in abscised leaves, compared to the values found in summer analysis was the result of the translocation of nutrients into storage organs (branches, trunks and roots), for the development of new tissues in the following spring. This behaviour is known for N (Munoz et al. 1993; Millard and Thomson 1989), however it is less studied for K and Zn (Marschner 1995). In our investigation the amount of N stored in perennial organ in autumn accounted for 38 (±15), 35 (±15) and 40 (±9) g N $plant^{-1}$, in compost, cow manure and mineral fertilized tree, respectively, that makes an amount of approximately 20 kg N ha^{-1} and provided a magnitude of nectarine tree N internal cycling. In fact, considering a total N requirement for peach of 100–150 kg N ha^{-1}, the fraction of re-cycling N, calculated for the nectarine under investigation was 20 % or less. The ability of mature peach to withdraw N during senescence was about 50 % of the total leaf N, similar to the values already found by El-Jendoubi et al. (2013).

The amount of K and Zn recycled was lower than N and accounted for approximately 5.7, 3.7 and 1.9 g K tree^{-1}, and 91, 27 and 24 mg Zn tree^{-1} for compost, cow manure and mineral fertilized trees, respectively. Actually, Marschner (1995) reported a significant remobilization of P, in perennial plants, in disagreement with our findings, that showed a constant leaf concentration of P during the season. It may be possible that part of P (specifically the amount up taken and accumulated into the leaves after the end of July) was re-translocated outside, and not measured by our approach. Another possibility is that the fraction of P used for remobilization in spring, as reported by Marschner (1995), was not from autumn leaf withdrawal but stored directly into the roots, trunk or branches. In our study leaf concentration of Ca, Fe and Mn increased until leaf senescence as confirmed in previous studies (Marschner 1995), with no difference among treatments. The concentration of Fe was almost three times higher in abscised than summer sampled leaves, showing that leaves are an active sink for Fe during the entire season. This behaviour is known to be promoted by a high leaf N, since high concentration of protein decreases Fe mobilization and translocation (Marschner 1995) along with the formation of the Fe chelators (Shi et al. 2012).

In our experiment the organic fertilization did not promote any accumulation of heavy metals in the soil after 13 years of continuous compost application. However, comparing the soil analysis at the beginning and at the end of the trail, we observed a slight increase of Cd, Cu and Cr and a decrease of Ni, Pb and Hg. While the increase of Cu in soil could be related to the use of copper based agrochemicals against pest and diseases, Cd and Cr are mainly produced by incinerator and by the combustion of fossil fuels. Since the experimental farm is situated next to the municipal incinerator of Ravenna in the most industrialized harbour area, the increase of these heavy metals could be due to environmental pollution. The decrease of Ni, Pb and Hg could be the consequence of, other than plant uptake, leaching. In a recent trial conducted on potted trees (Sorrenti, 2015) the leaching rate accounted for (in g year^{-1} ha^{-1}) Ni 47, Pb 97.2 and Hg 4.81 in compost treated plots and Ni 13.6, Pb 51.3 and Hg 2.85 for unfertilized control. However, after a nectarine orchard lifetime, heavy metals concentration is lower or similar (for Cu and Zn) to the average in Italian soils (Abollino et al. 2002; Facchinelli et al. 2001) and within the ranges observed in various areas worldwide (Alloway 1990; Kelepertzis 2014), meaning a low impact fertilization approach.

These results are in accordance with some earlier reports (Montemurro et al. 2005; Zhao and Duo 2015) that showed compost does not increase total heavy metals in soils; however, there are some other investigations remarking heavy metal accumulation problem in soils amended with compost. Ayari et al. (2010) reported, for example, that applications of 40 and 80 t ha^{-1} year^{-1} MSW compost increased soil total Cd, Cr, Cu, Ni, Pb and Zn concentrations. Similarly, Pinamonti et al. (1997) found that the use of compost increased both total and EDTA extractable forms of Cu, Zn, Ni, Cr, Cd, and Pb in the soil. In a study conducted in Turkey (Yuksel 2015), heavy metal contents of the soils increased by 14–278 % after application of 200 t ha^{-1} of compost if compared to the control treatment. These differences can be related to different application rates, cropping system, soil properties and compost quality. The composition of MSW used in this study included mainly kitchen organic wastes and green wastes from urban garden and park maintenance usually not rich in heavy metals. Other MSWs may also include a fraction of agri-food organic wastes, slurry or animal manure; so that it is important to use high quality compost. Even if the compost we used in the trial slightly increased soil total Pb concentrations, the concern about heavy metal accumulation in soil and consequently in tree fruits was not confirmed by our data, testifying the good quality of compost processing and final product. These results are supported by the DCA analysis that showed a separated, independent distribution of total Cu and Zn concentration in soil, in the leaves and in fruits as a result of the absence of relation between heavy metal collocation in soil and plant. In addition, the DTPA extractable soil fraction of Zn and Cu were not related with the leaf and fruit concentration. This response was expected because of the calcareous nature of soil, that sequestrates metals as already observed in a trial on pear (Toselli et al. 2008) and grape (Toselli et al. 2009) grown in a clay loam calcareous soil enriched with a Cu range from 0 to 1000 mg kg^{-1}.

Conclusions

In conclusion, the present investigation showed that, in the long term, the fertilizer management of fruit trees with organic fertilizers in which nutrients are not immediately available for root uptake (as it is for MSW compost and cow manure) can be a valuable alternative to traditional mineral fertilization of nectarine trees cultivated in the Italian Po Valley. In particular, the use of compost showed a better effect than cow manure in term of soil OM and N accumulation, without increasing mineral N soil availability over the tree requirement rate. At the same time, the high quality of MSW compost prevented any accumulation of heavy metal in soil and plant tissues. In intensive agricultural systems, compost can be introduced for its low cost and social benefits related to the recycling of organic wastes with no negative effects on the environment. Future research are required to evaluate the maximum rate of compost that can be supplied to orchard without negative effect on plants and soil and the correct compost C/N ratio in order to define the best practice for each soil condition and crop type.

References

Abollino O, Aceto M, Malandrino M, Mentasti E, Sarzanini C, Petrella F (2002) Heavy metals in agricultural soils from Piedmont, Italy. Distribution, speciation and chemometric data treatment. Chemosphere 49(6):545–557. doi:10.1016/S0045-6535(02)00352-1

Aguirre PB, Hinai YKA, Roper TR, Krueger AR (2001) Apple tree rootstock and fertilizer application timing affect nitrogen uptake. Hortic Sci 36:1202–1205

Alloway BJ (1990) The origins of heavy metals in soils. In: Alloway BJ (ed) Heavy metals in soils. Blackie, Glasgow and London, pp 29–39

Ayari F, Hamdi H, Jedidi N, Gharbi N, Kossai R (2010) Heavy metal distribution in soil and plant in municipal solid waste compost amended plots. Int J Environ Sci Technol 7:465–472. doi:10.1007/BF03326156

Baldi E, Toselli M, Scudellari D, Marangoni B (2004) La concimazione fogliare delle drupacee (Foliar fertilization of stone fruit). Inf Agrar 23:65–68

Baldi E, Toselli M, Marcolini G, Quartieri M, Cirillo E, Innocenti A, Marangoni B (2010) Compost can successfully replace mineral fertilizers in the nutrient management of commercial peach orchard. Soil Use Manag 26:346–353. doi:10.1111/j.1475-2743.2010.00286.x

Baldi E, Marcolini G, Quartieri M, Sorrenti G, Toselli M (2014) Effect of organic fertilization on nutrient concentration and accumulation in nectarine (*Prunus persica* var. nucipersica) trees: the effect of rate of application. Sci Hortic 179:174–179. doi:10.1016/j.scienta.2014.09.029

Burger M, Jackson LE (2003) Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biol Biochem 35(1):29–36

Carranca C (2012) Nitrogen use efficiency by annual and perennial crops. In: Lichtfouse E (ed) Farming for food and water security, vol 10. Springer, The Netherlands, pp 57–82. doi: 10.1007/978-94-007-4500-1_3

Chadwick D, Sommer S, Thorman R, Fangueiro D, Cardenas L, Amon B, Misselbrook T (2011) Manure management: implications for greenhouse gas emissions. Anim Feed Sci Technol 166:514–531. doi:10.1016/j.anifeedsci.2011.04.036

Diacono M, Montemurro F (2010) Long-term effects of organic amendments on soil fertility. A review. Agron Sust dev 30(2):401–422. doi:10.1051/agro/2009040

El-Jendoubi H, Abadía J, Abadía A (2013) Assessment of nutrient removal in bearing peach trees (*Prunus persica* L. Batsch) based on whole tree analysis. Plant Soil 369(1–2):421–437. doi:10.1007/s11104-012-1556-1

Facchinelli A, Sacchi E, Mallen L (2001) Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. Environ Pollut 114(3):313–324. doi:10.1016/S0269-7491(00)00243-8

FAO (2010) World references base for soil resources. http://www.fao.org/nr/land/soils/en/

Gallardo-Lara F, Nogales R (1987) Effect of the application of town refuse compost on the soil–plant system: a review. Biol Wastes 19:35–62. doi:10.1016/0269-7483(87)90035-8

Kelepertzis E (2014) Accumulation of heavy metals in agicultural soils of Mediterranean: insight from Angolida basin, Peloipponnese, Greece. Geoderma 221:82–90. doi:10.1016/j.geoderma.2014.01.007

Kersebaum KC, Steidl J, Bauer O, Piorr HP (2003) Modelling scenarios to assess the effects of different agricultural management and land use options to reduce diffuse nitrogen pollution into the river Elbe. Phys Chem Earth 28:537–545. doi:10.1016/S1474-7065(03)00090-1

Kingston HM (1988) Microwave assisted acid digestion of siliceous and organically-based matrices, Method 3052. U.S. Environmental Protection Agency IAG DWI-393254-01-0, Quarterly Report, January 1–March 31

Leita L, Petruzzelli G (2000) Metalli pesanti. In: Violante P (ed) Metodi di analisi chimica del suolo, chapter XI. Angeli, Milan, pp 1–18

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am J 42:421–428. doi:10.2136/sssaj1978.03615995004200030009x

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic press, London

Millard P, Thomson CM (1989) The effect of the autumn senescence of leaves on the internal cycling of nitrogen for the spring growth of apple trees. J Exp Bot 40(11):1285–1289. doi:10.1093/jxb/40.11.1285

Montemurro F, Maiorana M, Convertini G, Fornaro F (2005) Improvement of soil properties and nitrogen utilization of sunflower by amending municipal solid waste compost. Agron Sustain Dev 25:369–375. doi:10.1051/agro:2005038

Munoz M, Guerri J, Legaz F, Primo-Millo E (1993) Seasonal uptake of SN-nitrate and distribution of absorbed nitrogen in peach trees. Plant Soil 150:263–269. doi:10.1007/BF00013023

Nawab J, Khan S, Shah MT, Khan K, Huang Q, Ali R (2015) Quantification of heavy metals in mining affected soil and their bioaccumulation in native plant species. Int J Phytoremed 17(9):801–813. doi:10.1080/15226514.2014.981246

Neilsen D, Millard P, Neilsen GH, Hogue EJ (2001) Nitrogen uptake, efficiency of use, and partitioning for growth in young apple trees. J Am Soc Hortic Sci 126:144–150

Pinamonti F, Stringari G, Gasperi F, Zorzi G (1997) The use of compost: its effects on heavy metal levels in soil and plants. Res Conserv Recycl 21:129–143. doi:10.1016/S0921-3449(97)00032-3

Potter KM, Cubbage FW, Blank A, Schaberg RH (2006) Watershed-scale model for predicting nonpoint pollution risk in North Carolina. Environ Manag 34:62–74. doi:10.1007/s00267-004-0117-7

Rahman MA, Rahman MM, Reichman SM, Lim RP, Naidu R (2014) Heavy metals in Australian grown and imported rice and vegetables on sale in Australia: health hazard. Ecotoxicol Environ Safety 100:53–60. doi:10.1016/j.ecoenv.2013.11.024

Saunders WM, Williams EG (1955) Observations of the determinations of organic phosphorus in soils. J Soil Sci 6:254–267. doi:10.1111/j.1365-2389.1955.tb00849.x

Shi R, Weber G, Köster J, Reza-Hajirezaei M, Zou C, Zhang F, von Wirén N (2012) Senescence-induced iron mobilization in source leaves of barley (*Hordeum vulgare*) plants. New Phytol 195:372–383. doi:10.1111/j.1469-8137.2012.04165.x

Singh J, Kalamdhad AS (2013) Chemical speciation of heavy metals in compost and compost ammended soil—a review. Int J Environ Eng Res 2:27–37

Sorrenti G (2015) Biochar in perennial crops: nutritional, agronomical and environmental implications. PhD thesis, University of Bologna

Tagliavini M, Scudellari D, Marangoni B, Toselli M (1996) Nitrogen fertilization management in orchards to reconcile productivity and environmental aspects. Fert Res 43:93–102. doi:10.1007/978-94-009-1586-2_27

Toselli M, Baldi E, Marcolini G, Malaguti D, Quartieri M, Sorrenti G, Marangoni B (2008) Response of potted pear (*Pyrus communis* L.) trees to increasing Cu concentration in sandy and clay-loam soils. J Plant Nutr 31:2089–2104. doi:10.1080/01904160802459609

Toselli M, Baldi E, Marcolini G, Malaguti D, Quartieri M, Sorrenti G, Marangoni B (2009) Response of potted grapevines to increasing soil copper concentration. Aust J Grape Wine Res 15:85–92. doi:10.1111/j.1755-0238.2008.00040.x

Violante P (2000) Metodi di analisi chimica del suolo (Methods of soil chemical analysis). Franco Angeli, Milano, p 496

Volk M, Liersch S, Schmidt G (2009) Towards the implementation of the European Water Framework Directive? Lessons learned from water quality simulations in an agricultural watershed. Land Use Policy 26:580–588. doi:10.1016/j.landusepol.2008.08.005

Regione Emilia-Romagna http://agricoltura.regione.emilia-romagna.it/produzioni-agroalimentari/temi/disciplinari-produzione-integrata

Yuksel O (2015) Influence of municipal solid waste compost application on heavy metal content in soil. Environ Monit Assess 187(6):1–7. doi:10.1007/s10661-015-4562-y

Zhao S, Duo L (2015) Bioaccumulation cadmium, copper, zinc and nickel by weed species from municipal solid waste compost. Polish J Environ Stud 24:413–417. doi:10.15244/pjoes/28960