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Constructed wetland biomass for compost production: Evaluation of effects on crops and soil

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A B S T R A C T

This study investigates the suitability of *Phragmites australis* (reed) biomass deriving from a surface flow constructed wetland (CW) to produce three compost types: reed (**RC**), reed mixed + potato cuttings (**PC**) and reed + liquid anaerobic digestate (**DC**), to promote both resource circularity and soil carbon sequestration. The composts were tested over 60 days on lettuce at two levels in combination or not with NH_4NO_3 (at the same kg N ha^{-1} loading), along with NH_4NO_3 reference (**Chem**) and an unamended control (**Ctrl**). The plant tissue dry weight and N load was determined, and the N relative efficiency (N-RAE %) was calculated. On pot soil, total and labile carbon (TOC, C_L), along with the carbon management index (CMI) and $\delta^{13}C$ were evaluated. Pot test showed that $\mathbf{PC_{100}}$ yielded the best (g pot^{-1}) lettuce biomass (3.0) > $\mathbf{DC_{100}}$ and $\mathbf{RC_{100}}$ (2.5 and 1.6) ≈ chemical reference (3.8). A similar pattern was detected at 50% (g pot^{-1}): $\boldsymbol{PC_{50}}$ (2.9) > $\mathbf{DC_{50}}$ (2.7) > $\mathbf{RC_{50}}$ (2.4). N-RAE (%) reflected this pattern: $\boldsymbol{PC_{100}}$ (60) > $\boldsymbol{DC_{100}}$ (21) > $\boldsymbol{RC_{100}}$ (10) and $\boldsymbol{PC_{50}}$ (76) > $\boldsymbol{DC_{50}}$ (53) > $\boldsymbol{RC_{50}}$ (52). Pot soil analyses showed composts well performed in TOC and CMI, in comparison to Ctrl (+42% and +13%), suggesting a positive impact on soil C amelioration. No significant differences were observed for $\delta^{13}C$ distribution, suggesting the composts did not influence the microbic metabolism differently. These results indicated that the biomass harvested from the **CW**s can represent an interesting material for composting, combining carbon sequestration and nutrients recycling potential of these system, in addition to their wastewater treatment capacity.

1. Introduction

Human activities, including urban, agricultural and industrial processes, produce a number of wastes, including wastewater (Peters, 2011). In particular, agricultural drainage water (ADW) is a type of wastewater which contains different contaminants (e.g. fertilisers, pesticides) used in crop production and it represents a major non-point pollution source. Since it potentially contains various harmful compounds, it requires a proper treatment prior to the discharge in the water cycle (Braschi et al., 2022; Larsen et al., 2016). In facts, ADW pollutants have been found to cause various disturbances in the receiving ecosystems, including eutrophication, groundwater contamination, soil degradation, risks to human and animal health, and loss of biodiversity (Ansari and Gill, 2014; Johannesson et al., 2017).

One of the primary research areas in the effort to treat ADW are Nature-Based Solutions (NBSs), systems where natural processes are applied to remediate contaminated water resources (Nan et al., 2023). Amongst the NBSs, constructed wetlands (CWs) are an example of NBSs that leverage the interactions between selected plants, associated microorganisms and specific substrates for wastewater treatment from various sources (Mancuso et al., 2024; Milani et al., 2019; Parde et al., 2021). Particularly effective for treating ADW, CWs commonly host low-maintenance plants species with low fertilisation, tilling and irrigation needs (Parde et al., 2021). Nitrogen (N) and phosphorus (P) are two nutrients that are essential to soil health and fertility, and that can be retained by CWs from ADW through the aboveground biomass (Kasak et al., 2020; Li et al., 2021). To avoid their return in the effluent, regular harvest is suggested, and the collected CW biomass can be employed as alternative feedstock for biogas and biochar production, and for material preparation (Cui et al., 2022; Pinho and Mateus, 2023). Another possible option is to compost the harvested plants enabling nutrient reuse and recovery (Grigatti et al., 2012; Reyes-Torres et al., 2018). The result is a product that can benefit soil quality and fertility, and whose application can reduce the reliance on mineral fertilisers (Amelung et al., 2020; Pereira et al., 2022).

Fundamental to the dynamics of these nutrients, C is a useful

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parameter for assessing soil quality: its labile fraction (C_L), in facts, feeds the microbiota responsible for the nutrients cycling within the pedosphere. The distribution of C fractions, represented by the C management index (CMI), can thus be indicative of the soil functionality and health, and can provide useful information about the impact of different agricultural practices (Blair et al., 1995). Furthermore, through isotopic ^{13}C analysis and its abundance in comparison to ^{12}C, it is possible to evaluate the soil microbial activity, and to collect information supporting the nutrients dynamics study (Wang et al., 2015).

Within this context, the goal of the study was to evaluate the suitability of *Phragmites australis* (reed) aerial biomass derived from an ADW-treating CW, along with two other agricultural by-products, potato cuttings and anaerobic digestate, to produce soil amendments that can be suitable for the application in agriculture and that can be an alternative resource to chemical fertilisers, in line with former research (Arab et al., 2022; Kwarciak-Kozłowska, 2019; Pergola et al., 2020; Thu and Loan, 2024). Therefore, a *Lactuca sativa* (lettuce) pot test was performed in a controlled environment with different amendment application levels (ALs) to assess the compost capacity to influence and improve the crop growth, as well as its effect on soil characteristics.

2. Materials and methods

2.1. Study area

The test site was an experimental farm in the vicinity of Budrio village (Emilia Romagna Region, Italy) that also hosts a surface flow CW which treats ADW from the farm (Lavrnić et al., 2018). As specified by Regional Agency for Environmental Protection (ARPAE) from 30-year normal values, the area is characterised by a subtropical humid climate (Cfa) according to the Köppen classification of climate, with a mean annual temperature of around 13.7 °C and a mean annual rainfall of 771 mm, with most of precipitations during spring and fall. The soil of the CW establishment is Udifluventic Haplustept (Soil Survey Staff, 2014), while the system has an overall surface of 0.4 ha and a storage capacity of 1477 m^3, and hosts mainly *Phragmites australis*, but also *Iris* spp. and *Carex* spp. (Lavrnić et al., 2020a). This CW was extensively studied in past, focusing on the aspects such as water quality (Braschi et al., 2022; Buscaroli et al., 2024; Canet-Martí et al., 2022), hydraulic behaviour (Lavrnić et al., 2020b) and sustainability assessment (García-herrero et al., 2022). Due to its specific nature and big biomass availability, this study aimed to further explore the circularity of the system and its potential for compost production.

2.2. Compost preparation and analysis

As a raw compost material, fresh reed was cut from the CW, chopped to 2 cm and placed in ad-hoc constructed compost bins of 1 m^3. Three different composts were consequently produced: reed alone (compost "**RC**"); 25% (wt./wt.) reed added with 75% of potato cuttings (compost "**PC**") obtained from the same farm; and 63% (wt./wt.) reed added with 37% of digestate (compost "**DC**"). The proportions chosen for DC and PC were based on the suggested C:N ratio between 20 and 30 (Hu et al., 2020). The three systems were maintained at a proper moisture level and periodically mixed for 2.5 months. Once ready, the compost aliquots were air-dried at room temperature, ground to 2 mm and stored for further analyses and for the pot test. A commercial green waste compost ("**GWC**") was also used to provide a treatment reference.

Fresh compost samples were prepared according to the standard BS EN 13038:2011 for pH and electrical conductivity (EC) measurements. Total Solids (TS), volatile solids (VS) and oxygen uptake rate (OUR) were measured according to BS EN 13039:2011, BS EN 13040:2007 and BS EN 16087–1:2020 standards, respectively. TOC and total N (TN) of the dried and ball-milled samples were determined with a Flash 2000 Series Organic Elemental Analyser. The isotopic abundance of soil ^{13}C was determined with a DELTA V Advantage coupled mass spectrometer (Thermo Electrone Germany). The C_L soil fraction was measured according to the method of Blair et al. (Blair et al., 1995). Elemental composition was assessed following the procedure indicated by EPA (Campisano et al., 2017), with an Arcos-Ametek 160 nm ~ 780 nm ICP-OES. Soil Olsen P was measured on air-dried, milled soil samples according to the Olsen method readapted (Watanabe and Olsen, 1965).

2.3. Pot test

The soil used for the experiment was air-dried and ground to 2 mm. The characteristics are reported in Table 1. It was then mixed with the produced experimental composts, to meet 50 (AL_{50}) or 100% (AL_{100}) of the theoretical N required in its available form for crop cultivation, namely 280 kg ha^{-1} (Ghosh et al., 2019). Thirty pots of 2 L were prepared by adding sand as drainage layer, which was covered with soil until reaching a height of 20 cm.

Six of the pots were prepared with unamended soil (without compost addition), of which three were fertilised with a first half of NH_4NO_3 fertilising solution ("**Chem**" samples), and three were left unfertilised ("**Ctrl**" samples). Other twenty-four pots were filled with the soil-compost mixes, with the same substrate layering.

After planting the lettuce, the pots were randomly placed in a growth chamber, with controlled humidity (45%), temperature (22–26 °C) and light exposure (approximately 15,000 lx), and equally irrigated with tap water at regular intervals. The pots were randomly repositioned every 15–20 days in order to ensure homogeneous conditions for all the samples. After 30 days of growth, the AL_{50} pots were added with a chemical fertiliser to reach 280 kg N ha^{-1}, and the **Chem** pots received the second half of the N supplementation.

On the 60th day, the plants were harvested, and their aerial parts, roots and soil were separated for differentiated analyses. The plant parts were rinsed when necessary and dried in a ventilated oven at 60 °C for 2 days. The soil was placed in an aerated room and dried at room temperature (c.a. 22 °C). Once dried, the samples and the composts were ball-milled and collected.

2.4. Agronomic parameters calculation

N uptake, percentile Relative Agronomic Efficiency (% N-RAE) and percentile N Apparent Recovery Factor vs. the chemical control (% N-ARF) were calculated according to the following formulas.

N Uptake was calculated as shown in the Eq. 1.

$$N\ Uptake_{(i)} = Tissue\ TN_{(i)} \bullet Tissue\ dry\ weight_{(i)} \quad (1)$$

Where "*i*" is the considered sample; "Tissue TN" is the total N measured in either the aerial parts or the roots of the sample "*i*" (mg g^{-1}); tissue dry weight is the harvested and dried aerial or root part of the sample "*i*" (g).

N-RAE (%) was calculated as follows:

Table 1
Properties of the soil employed for the pot trial.

Parameter	Value
pH	8.25
EC	0.21 dS m^{-1}
Sand	25%
Silt	54%
Clay	21%
TOC	9.8 mg kg^{-1}
Organic Matter	1.69%
Total Carbonates	5.3%
Active Carbonates	2.2%
TN	0.9 mg kg^{-1}
Ammonia N	87.2 mg kg^{-1}
Nitric N	5.4 mg kg^{-1}
Olsen P	5.2 mg kg^{-1}
Cation Exchange Capacity	22.2 cmolc kg^{-1}

$$N-RAE_{(i)} = \frac{N\,uptake_{(i)} - N\,uptake_{(Ctrl)}}{N_{appl(i)}} \bullet 100 \quad (2)$$

Where:

N_{appl} is the N weight applied through chemical fertilisation (mg) to each "*i*" sample.

N-ARF (%) for the "*i*" sample was defined with Eq. 3:

$$N-ARF_{(i)} = \frac{N\,uptake_{(i)} \Big/ N_{appl(i)}}{average\ N\,uptake_{(ctrl)} \Big/ average\ N_{appl(chem)}} \bullet 100 \quad (3)$$

The CMI was calculated through Eq. 4:

$$CMI\ (\%) = CPI \bullet LI \bullet 100 \quad (4)$$

Where CPI (Carbon Pool Index) is calculated as:

$$CPI = \frac{TOC_{treated\ soil\ sample}\left(g\,kg^{-1}\right)}{TOC_{reference\ soil}\left(g\,kg^{-1}\right)}; \quad (5)$$

LI (Lability Index) is calculated as:

$$LI = \frac{L_{(i)}}{L_{(reference)}}, \quad (6)$$

Where:

L (Lability) is the ratio of C_L (g kg^{-1}) of the "*i*" sample, measured with the method of Blair et al., to C_{NL} (g kg^{-1}) of the same sample, measured as difference between TOC and C_L (Blair et al., 1995). The chemically fertilised set was used as reference. The δ^{13}C signature of the studied composts were calculated with Eq. 7:

$$\delta^{13}C\ (‰) = \left(\frac{R_{(i)}}{R_{(standard)}} - 1\right) \bullet 1000 \quad (7)$$

where R is the ^{13}C to ^{12}C ratio of the "*i*" sample.

2.5. Statistical analysis

The pots were prepared in triplicates for each treatment, in order to reduce the impact of the individual plant variability, and the samples for the analysis were collected and examined in triplicates to ensure statistical reliability. The acquired data were processed through ANOVA and Tukey's Honest Significant Difference tests, to evaluate the values distribution and the significance of their differences.

3. Results

3.1. Main composts characteristics

Table 2 reports the results of the composts characterisations. The smaller OUR of the commercial **GWC** indicated a lower microbial respiration and higher biological stability than the CW composts. These composts presented reciprocally similar values: in terms of microbial activity, **DC** resulted as the most stable, **RC** showed an intermediate value and **PC** was the least stable. **DC** and **RC** were characterised by the highest VS, and a similar trend was observed for the TOC content, with **RC** at the highest level, followed by **DC**, **GWC** and **PC**. TN and TP followed an opposite trend: **GWC** presented the highest content, analogous to **PC**; **DC** and **RC** were characterised by a lower, similar content. Consequently, the C:N ratio resulted significantly higher in **RC** (24) than in the other three composts (12−20). Regarding the δ^{13}C characterisation, no statistical differences were observed, with values ranging between −28.64 and −27.94‰.

3.2. Biomass yield and nitrogen balance

After the two-months period of the pot test (Fig. 1 and Fig. 2), the plant aerial parts, roots and soils were separated. The weights of the dry biomasses harvested, together with their N content, are reported in Table 3. The visual assessment identified the **Chem** samples as the healthiest specimens (less or no aerial part chlorosis and necrosis, highest leaves number and surface), followed by **GWC$_{100}$** and **PC$_{100}$**. Despite not having received any fertilisation, the **Ctrl** samples appeared in the same range of **PC$_{100}$** and **GWC$_{100}$**, and healthier than **DC$_{100}$** and **RC$_{100}$**. Amongst the AL_{100} samples, **PC$_{100}$** yielded the highest average biomass, reaching the same range of the **Chem** reference and **GWC$_{100}$**. It was followed by **DC$_{100}$**; **RC$_{100}$** showed the worst average biomass yield, lower than **DC$_{100}$**. Considering the AL_{50}, the potato-reed compost mix resulted again to be the most performing on average, with **PC$_{50}$** and **DC$_{50}$** yielding even more than the standardised **GWC$_{50}$** and remaining nearby the **Chem** range. **RC$_{50}$** showed the same yield as **GWC$_{50}$**.

N-ARF and N-RAE are reported in Table 4**. Chem** tissues showed the highest N uptake value amongst the unamended references. Amongst the AL_{100} amended samples, the detected trend is the following, from the highest to the lowest values: **GWC$_{100}$**, **PC$_{100}$**, **DC$_{100}$**, **RC$_{100}$**. At AL_{50}, potato-reed mix compost showed again the highest level, higher than **GWC$_{50}$**, that resulted similar to **DC$_{50}$** and **RC$_{50}$**. Considering N-ARF, all the treatment performed worse than **Chem** both at AL_{100} and AL_{50}. Regarding N-RAE, **PC** performed the best in both the application levels, outscoring the other treatments, especially at AL_{50}.

3.3. Soil carbon fractioning

The results of the analyses for TOC, C_{NL} and C_L are reported in Table 5. At the end of the two months-pot trial, the unfertilised and chemical references presented a TOC content similar to the starting soil. Regarding the AL_{100} series, **RC$_{100}$** showed the highest TOC content, **DC$_{100}$** and **PC$_{100}$** were statistically equal, while **GWC$_{100}$** had a TOC content slightly lower than the others. The samples of AL_{50} series presented an overall lower TOC content in respect to AL_{100}, with comparable values amongst samples that ranged from 16.85 to 17.39 g kg^{-1}. Regarding C_{NL}, the **Chem** and **Ctrl** references were in the range between 13.30 and 14.22 g kg^{-1} (93.4–94.7 TOC). At AL_{100}, **RC$_{100}$** showed a significantly higher content (20.22 g kg^{-1}, 94.7% TOC), while **DC$_{100}$**, **PC$_{100}$** and **GWC$_{100}$** presented similar levels, between 18.66 and 19.09 g kg^{-1} (94.7–95.0% TOC). At AL_{50}, the amended samples showed no significant difference and ranged between 15.39 and 16.56 g kg^{-1} (94.7–95.3% TOC). Considering the C_L concentrations, the unfertilised references showed no statistical difference, with **Ctrl** accounting for 0.80 g kg^{-1} (5.3% TOC). **Chem** had an average value of 0.90 g kg^{-1} C_L (6.6% TOC). Amongst the amended samples, **RC$_{100}$** presented the highest C_L fraction (1.13 g kg^{-1}, 5.3% TOC), significantly different than

Table 2
Main characteristics of the compared composts.

Compost	pH	EC	OUR	TS	VS	TOC	TN	TP	C:N	δ^{13}C
	–	dS m^{-1}	(mmol O_2 kg^{-1} OM h^{-1})	(%)	(%)	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})		(‰)
DC	7.88	0.35	15	41.1	86.2	335	17.6	1.5	20	−27.94
PC	8.62	0.55	22	36.4	63.1	243	20.1	2.2	12	−28.19
RC	7.65	0.51	18	48.4	88.9	412	17.0	1.2	24	−28.56
GWC	8.91	0.13	7	59.0	52.5	308	20.8	3.1	15	−28.64

DC: digestate-reed compost; **PC**: potato-reed compost; **RC**: reed only compost; **GWC**: green waste compost.

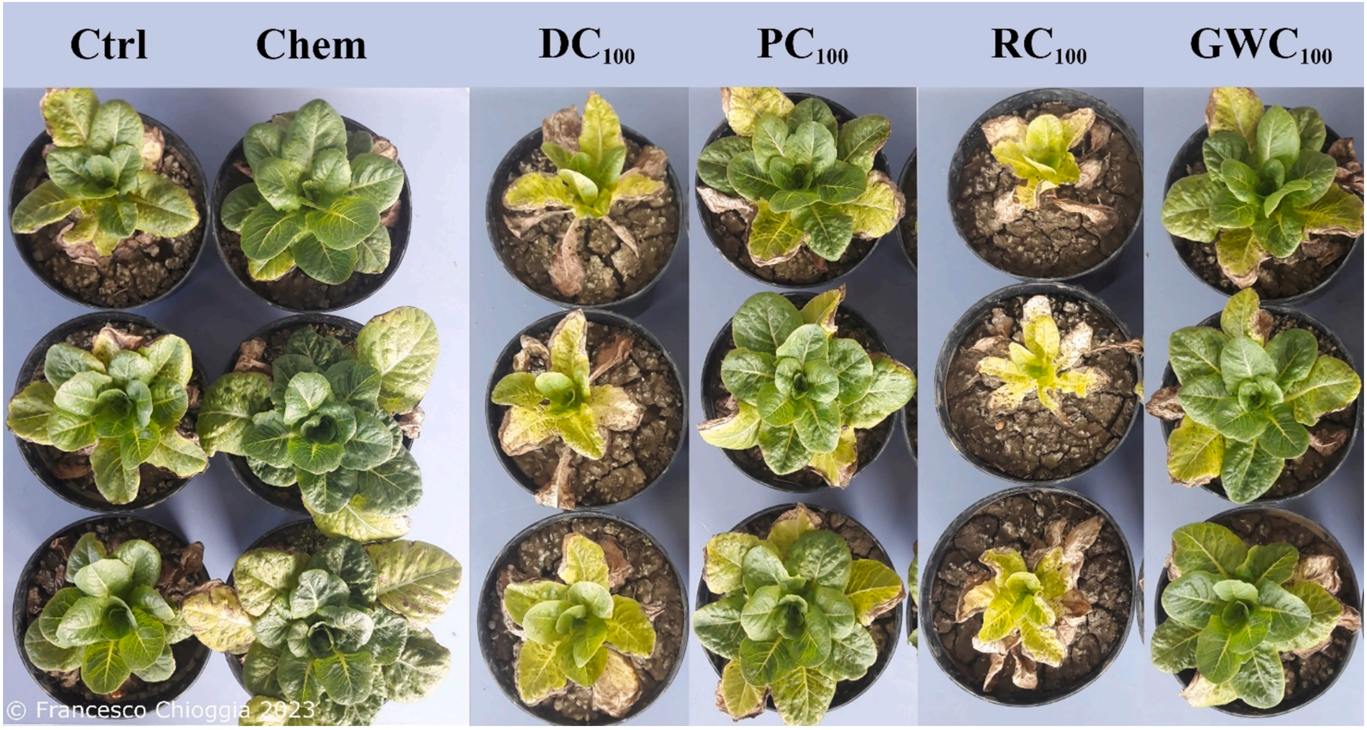

Fig. 1. Status of the plants at the end of the pot test, 100% application level. **Ctrl**: unamended control soil; **Chem**: chemical reference; **DC**: digestate-reed compost; **PC**: potato-reed compost; **RC**: reed only compost; **GWC**: green waste compost. Acronym subscript refers to amendment application level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

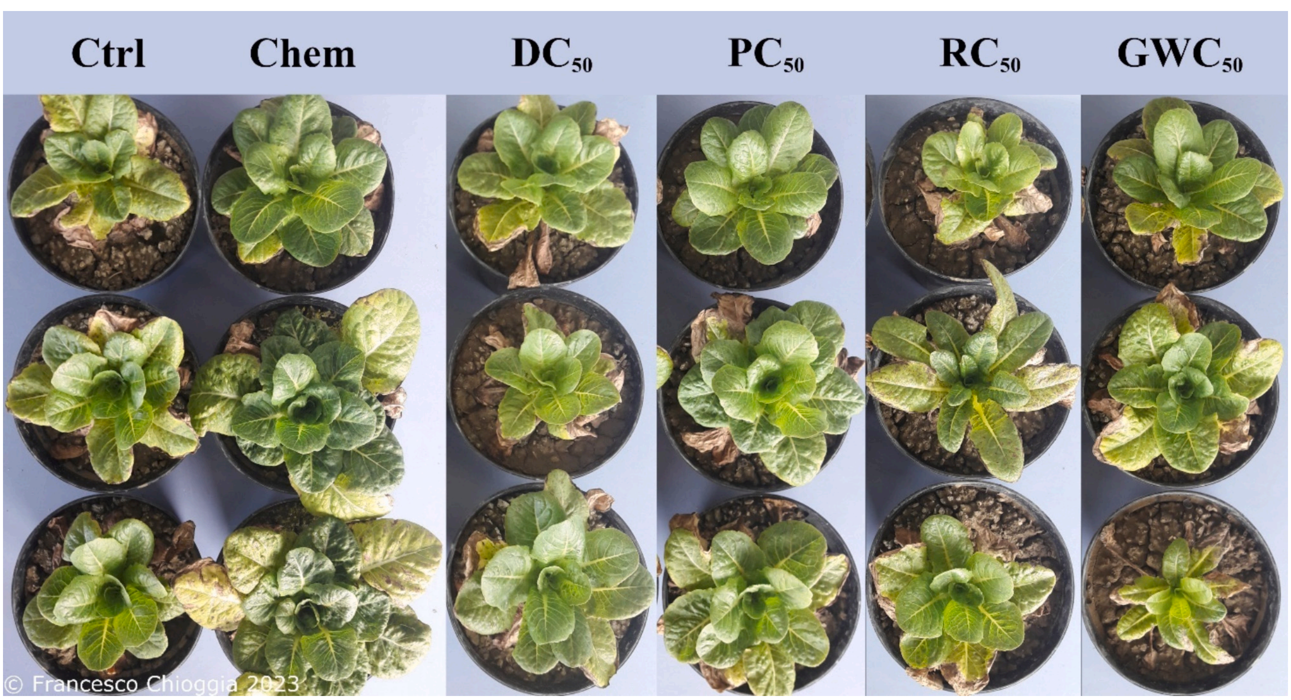

Fig. 2. Status of the plants at the end of the pot test, 50% application level. **Ctrl**: unamended control soil; **Chem**: chemical reference; **DC**: digestate-reed compost; **PC**: potato-reed compost; **RC**: reed only compost; **GWC**: green waste compost. Acronym subscript refers to amendment application level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the other treatments, that ranged between 0.93 (5.0% TOC) and 11.1 (5.6% TOC). However, considering the relative concentration of C_L in relation to TOC, the samples presented similar values, ranging between 5.0 and 5.6 g kg^{-1}. Regarding the AL_{50} group, the samples resulted more clustered, with no significative difference in between and with values ranging between 0.82 and 0.88 g kg^{-1} (4.7–5.3% TOC), with **PC_{50}** and **GWC_{50}** on the lower level of the range. Regarding the CMI, in reference to **Chem**, the **Ctrl** samples presented an index of 79%. Considering the AL_{100} series, **DC_{100}**, **RC_{100}** and **GWC_{100}** had similar values, higher than **Chem** and ranging between 108% and 113% while **PC_{100}** presented the

Table 3
Biomass yield and N content of leaves and roots of the harvested plants collected at the end of the pot trial from the pots cultivated at 0%, 50% and 100% organic amendment application levels.

Treatment	Aerial Parts Biomass	Roots Biomass	Total Biomass	Aerial Parts N	Roots N	Total N
	($g\ pot^{-1}$)	($g\ pot^{-1}$)	($g\ pot^{-1}$)	($mg\ pot^{-1}$)	($mg\ pot^{-1}$)	($mg\ pot^{-1}$)
Ctrl	2.1^{ac}	0.5^{ab}	2.6^{ab}	39.0^{bc}	3.9^{ac}	42.8^{bc}
Chem	3.1^{a}	0.7^{ab}	3.8^{a}	76.5^{a}	7.2^{ab}	83.7^{a}
DC_{100}	1.6^{ac}	0.9^{a}	2.5^{ab}	14.3^{cd}	5.3^{ac}	19.5^{cd}
PC_{100}	2.8^{ab}	0.3^{b}	3.0^{ab}	40.7^{bc}	1.8^{c}	42.5^{bc}
RC_{100}	0.9^{c}	0.7^{ab}	1.6^{b}	6.6^{d}	4.6^{ac}	11.2^{d}
GWC_{100}	2.3^{ac}	0.5^{ab}	2.8^{ab}	39.0^{bc}	4.9^{ac}	44.0^{bc}
DC_{50}	2.0^{ac}	0.7^{ab}	2.7^{ab}	40.8^{bc}	2.7^{bc}	43.4^{bc}
PC_{50}	2.4^{ac}	0.5^{ab}	2.9^{ab}	57.8^{ab}	4.2^{ac}	62.1^{ab}
RC_{50}	1.8^{ac}	0.6^{ab}	2.4^{ab}	39.5^{bc}	4.3^{ac}	43.8^{bc}
GWC_{50}	1.6^{bc}	0.8^{a}	2.4^{ab}	40.2^{bc}	7.7^{a}	47.9^{b}

Ctrl: unamended control soil; **Chem**: chemical reference; **DC**: digestate-reed compost; **PC**: potato-reed compost; **RC**: reed only compost; **GWC**: green waste compost. Acronym subscript refers to amendment application level. One-way ANOVA was applied to the data; for each parameter, the superscript letters indicate statistically different averages according to Tukey HSD test ($P < 0.05$).

Table 4
Tissues N apparent recovery fraction (N-ARF) and relative agronomical efficiency (RAE) of the harvested plants leaves, collected from the pots cultivated at 0%, 50% and 100% organic amendment application levels.

Treatment	Leaves		Roots	
	N-ARF	N-RAE	N-ARF	N-RAE
	(%)	(% vs Chem)	(%)	(% vs Chem)
Chem	20.6^{a}	–	1.9^{a}	–
DC_{100}	-15.3^{cd}	21.0^{bc}	0.9^{ab}	82.1^{ab}
PC_{100}	1.1^{bc}	59.9^{a}	-1.1^{b}	24.9^{b}
RC_{100}	-20.0^{d}	9.8^{c}	0.4^{ab}	70.8^{ab}
GWC_{100}	0.04^{bc}	57.4^{a}	0.6^{ab}	68.3^{ab}
DC_{50}	1.0^{bc}	53.3^{ab}	-0.7^{b}	41.3^{ab}
PC_{50}	10.4^{ab}	75.6^{a}	0.2^{ab}	58.6^{ab}
RC_{50}	0.3^{bc}	51.6^{ab}	0.3^{ab}	67.0^{ab}
GWC_{50}	0.7^{bc}	52.5^{ab}	2.1^{a}	106.3^{a}

Ctrl: unamended control soil; **Chem**: chemical reference; **DC**: digestate-reed compost; **PC**: potato-reed compost; **RC**: reed only compost; **GWC**: green waste compost. Acronym subscript refers to amendment application level. One-way ANOVA was applied to the data; for each parameter, the superscript letters indicate statistically different averages according to Tukey HSD test ($P < 0.05$).

lowest value. The samples resulted more clustered at AL_{50}, with no significant difference between **DC_{50}**, **RC_{50}** and **PC_{50}**, and with values ranging between 81 and 87%. **GWC_{50}** presented the lowest value, 76%.

3.4. Soil ^{13}C signature

As reported in Fig. 3, **Ctrl** and **Chem** presented a statistically similar isotopic $\delta^{13}C$ signature (−24.12 and −24.13‰, respectively). The AL_{100} samples were found to have a significant depletion of ^{13}C: **GWC_{100}** presented the lowest value (−25.50‰), followed by **RC_{100}** (−25.36‰), **PC_{100}** (−25.23‰) and **DC_{100}** (−25.04‰). The chemical fertilisation applied to AL_{50}, caused the samples to have a lower depletion in comparison to the unamended **Ctrl**, with no significant reciprocal difference and values ranging between −24.78 and −24.52‰.

Fig. 3 shows the $\Delta^{13}C$ in respect to **Chem** as well. **Ctrl** resulted to have no significant difference in respect to **Chem**, with a $\Delta^{13}C$ of 0.01. All the amended samples presented a negative $\Delta^{13}C$, with the AL_{100} series showing lower average values, and no significant differences between each other, ranging between −1.37 and −0.91. Similarly, at AL_{50}, no significant difference was recorded, with average values higher than AL_{100} group and ranging between −0.65 and −0.39.

Table 5
Results for the analyses of total organic carbon (TOC), non-labile (C_{NL}), labile carbon (C_L) and carbon management index (CMI) of the soil samples collected at the end of the pot trial. Soil total nitrogen (TN) and plants to soil N:N ratio are also reported.

Treatment	TOC	C_{NL}	C_L	CMI	TN	N:N
	($g\ kg^{-1}$)	($g\ kg^{-1}$)	($g\ kg^{-1}$)	(%)	($g\ kg^{-1}$)	(Tissues to Soil)
Ctrl	14.83^{d}	14.22^{d}	0.80^{b}	79^{cd}	1.88^{d}	13.80^{a}
Chem	15.06^{d}	13.99^{d}	0.90^{ab}	100^{ac}	1.92^{d}	19.78^{ab}
DC_{100}	19.76^{ab}	18.66^{ab}	1.11^{ab}	110^{a}	2.16^{ab}	11.62^{b}
PC_{100}	20.09^{ab}	19.09^{ab}	0.93^{ab}	99^{ad}	2.28^{ab}	13.29^{ab}
RC_{100}	21.35^{a}	20.22^{a}	1.13^{a}	112^{a}	2.22^{a}	7.30^{ab}
GWC_{100}	19.37^{ac}	18.94^{ac}	1.01^{ab}	108^{ab}	2.19^{a}	12.86^{b}
DC_{50}	16.85^{cd}	15.97^{bd}	0.88^{ab}	87^{bd}	2.03^{bcd}	13.47^{ab}
PC_{50}	17.39^{bd}	16.56^{bd}	0.82^{b}	81^{cd}	2.12^{cd}	13.86^{ab}
RC_{50}	16.51^{cd}	15.64^{cd}	0.87^{ab}	86^{cd}	1.95^{abc}	12.46^{b}
GWC_{50}	15.51^{d}	14.73^{d}	0.83^{b}	76^{d}	1.96^{d}	12.25^{ab}

Ctrl: unamended control soil; **Chem**: chemical reference; **DC**: digestate-reed compost; **PC**: potato-reed compost; **RC**: reed only compost; **GWC**: green waste compost. Acronym subscript refers to amendment application level. One-way ANOVA was applied to the data; for each parameter, the superscript letters indicate statistically different averages according to Tukey HSD test ($P < 0.05$).

3.5. Soil phosphorus fractioning

Table 6 reports the Olsen P assessed in the soils collected after the two months pot trial. The unfertilised **Ctrl** and the **Chem** reference resulted to have, respectively, the lowest and the highest labile Olsen P content. Regarding AL_{100}, **GWC_{100}** presented the highest value, similar to **PC_{100}**, **DC_{100}** a slightly lower and **RC_{100}** presented the lowest value. Concerning the AL_{50} samples, **RC_{50}** was characterised by the highest Olsen *P* value, significantly higher than **GWC_{50}**. **DC_{50}** and **PC_{50}** presented similar values.

4. Discussion

4.1. Main composts characteristics

The different materials employed for composting, namely the reed, the potato cuttings and the digestate, played a critical role on the final characteristics of the organic products which was in line with previous research (Oviedo-Ocaña et al., 2019). In particular, the substrates obtained by composting reed alone (**RC**) and reed mixed with the digestate (**DC**) showed higher OM, TOC and C:N ratio than the **PC** and **GWC**. All the products microbial stability (OUR) complied with the maximum threshold imposed by the European Fertiliser Regulation of 25 mmol O_2 kg^{-1} organic matter h^{-1} and could, therefore, be considered as safe for agricultural applications (Council of the European Union, 2019).

4.2. Biomass yield and nitrogen balance

The higher C:N ratios could have caused an unbalance in the nutrient dynamics within the soil of **RC_{100}** and **DC_{100}**, which showed worse health, biomass yield and N uptake levels in comparison to **PC_{100}** and **GWC_{100}**. This is consistent with former studies, where compost C:N ratios higher than 20 caused N immobilisation by microorganisms and lead to a decreased biomass production, while lower values were shown to favour microbial N mineralisation and, consequently, higher bioavailability and biomass outputs (Brust, 2019; van der Sloot et al., 2022). In facts, a reduced N availability was shown to cause biomass reduction due to the plants diversion from development towards survival (Mu and Chen, 2021). This aspect is also reflected by the patterns observed for N-ARF and N-RAE, and by the ratio between the tissue N and the soil N content in relation to the C:N ratio of the applied

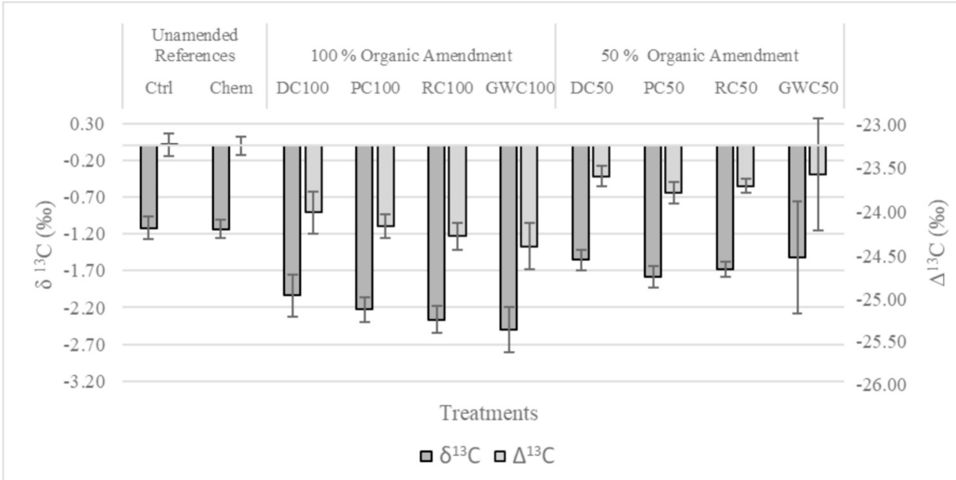

Fig. 3. δ ^{13}C and Δ^{13}C of the soil samples at the end of the pot tests. **Ctrl**: unamended control soil; **Chem**: chemical reference; **DC**: digestate-reed compost; **PC**: potato-reed compost; **RC**: reed only compost; **GWC**: green waste compost. The number following each acronym refers to amendment application level. Error bars indicate standard deviation of the mean.

Table 6
Soil available phosphorus and percentage of available phosphorus in relation to the total phosphorus recorded at the end of the two months pot trial, from the pots cultivated at 0%, 50% and 100% organic amendment application levels.

Treatment	Olsen P	TP %
	(g kg^{-1})	(%)
Ctrl	5.8^{d}	1.1^{cd}
Chem	16.5^{a}	2.8^{a}
DC$_{100}$	9.0^{cd}	1.6^{bd}
PC$_{100}$	10.5^{bd}	1.8^{bd}
RC$_{100}$	6.0^{d}	1.1^{d}
GWC$_{100}$	12.1^{ac}	2.02^{ac}
DC$_{50}$	6.5^{cd}	1.2^{bd}
PC$_{50}$	7.0^{cd}	1.2^{bd}
RC$_{50}$	15.5^{ab}	2.8^{a}
GWC$_{50}$	11.0^{bc}	2.0^{ab}

Ctrl: unamended control soil; **Chem**: chemical reference; **DC**: digestate-reed compost; **PC**: potato-reed compost; **RC**: reed only compost; **GWC**: green waste compost. Acronym subscript refers to amendment application level: "100" corresponds to AL_{100}, "50" corresponds to AL_{50}. One-way ANOVA was applied to the data; for each parameter, the superscript letters indicate statistically different averages according to Tukey HSD test ($P < 0.05$).

composts. It emerged that tissues-to-soil N ratio tended to increase with lower compost starting C:N ratio, suggesting a higher bioavailability with lower ratios, as previously stated. (Table 2 and Table 5).

The chemical supplementation of the AL_{50} samples seemingly boosted the health of plants, that produced aerial parts in similar quality and quantity and that presented similar levels of N uptake. An exception was **PC$_{50}$**, that presented higher levels in all the considered parameters, arguably due to the lower C:N ratio (− 26.7%) (van der Sloot et al., 2022). Chemical N fertilisation in addition to organic amendment, especially with high C:N ratios, was shown to shift the plants dependence from the microbial community composition towards the soil composition. In facts, AL_{50} samples were more clustered, since the chemical N supplementation arguably reduced the variability caused by the different amendment composition, rendering the plants more dependent upon the soil composition (Bossolani et al., 2023). Noteworthy, such supplementation produced specimens with outputs similar to the **Chem** samples: a partial substitution of the chemical fertiliser with composted biomass can induce yields comparable to a chemical-only fertilisation, thus presenting the opportunity to reduce the consumption of mineral resources and to increase the overall sustainability (Jin et al., 2022).

4.3. Soil carbon fractioning

The rise observed in C content within the amended samples (between 2% and 48% in relation to the **Ctrl** soil) indicated the positive effect of employing reed as compost feedstock in terms of C sequestration and soil enrichment in TOC content, especially in contrast to a chemical-only fertilisation, as shown by previous research (Wu et al., 2023). Most of the amended samples (except for **RC$_{50}$** and **GWC$_{50}$**) presented higher TOC and CMI than the unamended soils. This was in accordance with previous research, underlining the sustainability of CWs not only as a remediation technology, but also as carbon-mitigating systems, which allow the sequestration of atmospheric C and integration into the pedosphere (Cooper and DeMarco, 2023; Wijesekara et al., 2021).

Despite the differences observed in the TOC concentrations, the samples had similar C_L vs. TOC percentages, that ranged between 5.0% and 5.6%. The organic C added through amendment apparently presented a low labile fraction, and did not seem to contribute to almost any extent to the labilisation of the overall C. Conversely, it was observed that the chemical fertilisation of the **Chem** samples somewhat increased the C_L fraction in comparison to the other treatments, presenting a 27% higher relative C_L content, suggesting that a high chemical supplementation may render the already present C more bioavailable (Mayer et al., 2022). These aspects should be further explored, e.g. by studying the effect of different organic-chemical fertiliser proportions on the labilisation of the carbon, especially in relation to the added TOC. In contrast to the previously presented results, regarding the plants health and N management, **PC** and **GWC** soil samples featured lower CMI values than **DC** and **RC** samples. The soils amended with **DC** and **RC** could have been characterised by a higher C stability but a lower N bioavailability, due to their C:N ratio, as discussed before. In fact, since CMI is an index that focuses on the soil health, it does not consider other critical factors (e.g. nutrients bioavailability). Consequently, even at high CMI, the plants growth conditions may be sub-optimal, e.g. due to N availability reduction caused by microbial immobilisation (Brust, 2019; van der Sloot et al., 2022). On the other hand, this problem could be addressed by preparing the amendments of different composition. In particular, it would be important to reach lower C:N ratios, that can facilitate N labilisation and, consequentially benefit plants nutrition (Brust, 2019; van der Sloot et al., 2022).

4.4. Soil ^{13}C signature

Considering the negligible variation of δ^{13}C amongst the samples within each application level, it can be hypothesised that the applied composts did not particularly influence the biotic metabolism within the soil. This is consistent with the starting statistically similar compost $\delta^{13}C$ values, as well as with the lower isotopic abundance found for the AL_{50} samples when compared to the AL_{100} ones. The difference observed in comparison to the baseline and between the two application levels are probably related to the addition of C through the composts and not to differences in the biotic metabolism (Menichetti et al., 2013).

4.5. Soil phosphorus fractioning

P_L distribution reflected the pattern observed for the plant health and N management, with $\mathbf{PC_{100}}$ and $\mathbf{GWC_{100}}$ presenting the highest Olsen P content amongst the amended samples. This was arguably another aspect that could have contributed to such plants performances and have favoured their metabolism (Lizcano-Toledo et al., 2021). In contrast to the labile vs. total C (C_L vs. TOC) ratios, the labile vs. total P (Olsen P vs. TP) ratios followed the distribution of Olsen P itself, indicating that the P labile fraction remained apparently unaltered and proportional to the TP variation observed *ex post*. This might be related to aspects analogous to the ones influencing the C lability, as previously observed. Chemical N fertilisation may have promoted the internal soil dynamics, promoting the labilisation of the mineralised nutrients and a better absorption capacity of plants (Cui and Delgado, 2021).

5. Conclusions

The agricultural sector is one of the most important polluters of the water resources and constructed wetlands (CWs) are a valid method to treat surface runoff or drainage water from agricultural areas. One of the most common plants applied in CWs, reed was found to be unfit for direct application, if composted alone, requiring a partial chemical fertilisation. While a reed-digestate mixture showed results similar to the reed-only, potato cuttings seemed to be a promising material for increasing the compost quality, yielding healthier lettuce specimens, both in appearance and in terms of N management.

The reed-only and reed-digestate composts presented higher values (labile C and C Management Index) than the reed-potato mix. This aspect, however, did not account for other fundamental factors for plants growth, such as N. A partial chemical N fertilisation granted a considerable boost for all the amended samples, indicating a possibility to use CW-derived organic compost as a partial substitution of the chemical fertilisation and as a way for C relocating from the atmosphere into the soil. Additional analyses are suggested to explore further conditions, such as different composting and cultivation times, reed-wastes mixes and organic vs. chemical proportions, as well as to better comprehend the dynamics taking place within the soil amongst different components (e.g. microorganisms, plants, soils, nutrients).

6. Glossary

^{13}C isotope: naturally occurring carbon isotope useful for the determination of the carbon conservation and stability within soil (Inácio et al., 2018).

Agricultural drainage water: water deriving from precipitations upon and irrigation of cultivated crops, usually collecting in perimetral ditches surrounding the cultivated areas (Braschi et al., 2022).

Apparent Recovery Fraction, nitrogen (N-ARF): plants uptake of nitrogen, calculated in reference to a control sample, e.g. unfertilised, and employed for evaluating the impact of different treatments (Santos et al., 2018).

Bioavailability: availability of a certain element or compound to be readily absorbed and utilised by organisms, generally plants and microbes.

Carbon Management Index (CMI): index calculated from the soil Carbon Pool Index (CPI) and Lability Index (LI), which is used to evaluate soil health in terms of carbon storage and availability (Blair et al., 1995).

Constructed wetland: an engineered system where selected plants, their symbiotic microorganisms and particular substrates are applied for the treatment of polluted water or soil (Parde et al., 2021).

Carbon Pool Index (CPI): index calculated as ratio of total organic carbon (TOC) quantified in a sample soil and a reference, e.g. a chemically fertilised soil (Blair et al., 1995).

Green waste compost: compost produced from plants parts, in particular from plants and grasses cleaning and pruning byproducts, originating from sources such as municipal parks, domestic dwellings and gardens (Reyes-Torres et al., 2018).

Labile C: readily oxidisable carbon fraction, determined e.g. through the permanganate oxidisation method, present in a soil and available to organisms for their metabolism, also in function of the total organic carbon (see "Total Organic Carbon") (Blair et al., 1995).

Labile P: phosphorus fraction readily available to organisms for their metabolism, determined e.g. through bicarbonate extraction (Watanabe and Olsen, 1965).

Lability (carbon): ratio between the labile carbon present in a soil and the total organic carbon content of the same soil (Blair et al., 1995).

Lability Index (LI): ratio between the carbon lability of a sample soil and the carbon lability of a reference, e.g. a chemically fertilised soil (Blair et al., 1995).

Relative Agronomical Efficiency, nitrogen (N-RAE): index that compares the agronomic effects of an organic fertiliser with the effects of a chemical fertiliser, with focus on nitrogen (Santos et al., 2018).

Soil Organic Carbon (SOC): refer to "Total Organic Carbon (TOC)".

Total Organic Carbon (TOC): total carbon present in a sample and bound to organic molecules, directly influencing soil chemical, physical and biological characteristics, also in function of its labile fraction (see "Labile C") (Vieira et al., 2007).

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CRediT authorship contribution statement

Francesco Chioggia: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marco Grigatti:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Stevo Lavrnić:** Writing – review & editing, Investigation, Conceptualization. **Attilio Toscano:** Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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