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ORIGINAL RESEARCH

Belowground biomass C outweighs soil organic C of perennial energy crops: Insights from a long-term multispecies trial

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Abstract

The cultivation of perennial energy crops (PECs) couples the production of lignocellulosic biomass to the provision of multiple ecosystem services, such as the reduction of greenhouse gas emissions and the mitigation of climate change through carbon (C) sequestration in soil. Though C sequestration in soil by PECs has been widely studied, the contribution of their belowground biomass (BGB) to soil C sequestration and their influence on soil nitrogen (N) storage potential has received very little attention. In this study, C and N stocks in soil and BGB fractions (plant belowground organs and fine roots) were measured for six PECs (*Populus* spp. 'Poplar', *Robinia pseudoacacia* 'Black locust', *Salix* spp. 'Willow', *Arundo donax* 'Giant reed', *Miscanthus* × *giganteus* 'Miscanthus' and *Panicum virgatum* 'Switchgrass') grown on marginal soil, 11 years after establishment. All PECs had a higher soil organic carbon (SOC) stock and soil total nitrogen (STN) stock than arable land in the top (0–10 cm) soil layer. In this same top layer, woody crops had the highest SOC stock. The increase in SOC under PECs led to increased soil porosity in the top-soil layer. On average, 43% of the belowground C stock of PECs was allocated in the plant belowground organs (PBO; i.e. in the rhizomes of herbaceous PECs and the stump for woody PECs). Giant reed had the highest C stock in PBO, whereas switchgrass the lowest (22.7 vs. 5.9 Mg C ha^{-1}). On the contrary, switchgrass had the highest C stock in fine roots. Giant reed had the highest belowground C stock (sum of soil and BGB contribution) and black locust the highest belowground N stock. After 11 years of PEC cultivation, 68% of the belowground C stock was allocated in the BGB, and 32% was as SOC.

KEYWORDS

Arundo donax, belowground biomass, C sequestration, fine roots, *Miscanthus* × *giganteus*, *Panicum virgatum*, perennial energy crops, *Populus* spp., *Robinia pseudoacacia*, *Salix* spp.

1 | INTRODUCTION

The production of renewable energy from biomass crops has been one of the key components of the European policies in the last 30 years, for reducing greenhouse gases (GHGs) emission and achieving the renewable energy targets for 2030 (European Commission, 2017). Energy crops, and particularly perennial energy crops (PECs), have been at the centre

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of researchers' interest in the last 20 years for their high yield potential and lignocellulose biomass quality (Alexopoulou et al., 2015; Amaducci et al., 2017; Amaducci & Perego, 2015; Larsen et al., 2018; Lewandowski et al., 2003; Manzone et al., 2014). In addition, PECs have been considered a promising option to mitigate the effect of GHGs (Clifton-Brown et al., 2007; Gelfand et al., 2013; Schweier et al., 2017), to support bio-based economy (Lewandowski et al., 2003), and for the provision of multiple ecosystem services (Asbjornsen et al., 2014; Ferrarini et al., 2016, 2017, 2020; Kay et al., 2019). The sustainability of PECs lies in the possibility of extending the above-mentioned benefits, allocating the cultivation of these low-input crops on poor and marginal soils, excluding conflicts between food and energy productions and limiting the effects of indirect land use change. At a European level, 257 Mha of land has been classified as poor or very poor based on the Muencheberg soil quality rating system (SQR; Mueller et al., 2014) and approximately 58.2 Mha of these are suitable for PECs cultivation (Gerwin et al., 2018). Many studies have shown that herbaceous and woody PECs could contribute to soil carbon (C) storage through continuous addition of aboveground and belowground residues and reduced soil disturbance (Agostini et al., 2015; Chimento et al., 2016; Chimento & Amaducci, 2015; Rowe et al., 2016). In addition, it has been demonstrated that PECs have low nitrogen (N) and water requirements, despite their high-level biomass production (Alexopoulou et al., 2015; Amaducci et al., 2017; Arundale, Dohleman, Voigt, et al., 2014; Clifton-Brown et al., 2001). This is a consequence of (i) high nutrient and water absorption efficiency of deep-rooting systems (Cadoux et al., 2012; Chimento & Amaducci, 2015), (ii) high absorbed nutrient use efficiency, (iii) significant nutrient cycling between the reproductive organs and aerial biomass, (iv) nutrient recycling before harvest through leaf fall and (v) possible contribution of N fixation by bacteria (Cadoux et al., 2012). For these reasons, the cultivation of PECs on marginal lands matches two important environmental sustainability goals: soil quality improvement enabling the recovery of degraded lands, and the production of environmentally sustainable biomass for multiple uses.

PECs can allocate high amounts of atmospheric C in the belowground biomass (BGB; Chimento & Amaducci, 2015; Monti et al., 2012; Monti & Zatta, 2009) thanks to the deep and dense fine rooting systems (Chimento & Amaducci, 2015). As a result of this high C input to the soil (Agostini et al., 2015; Klimešová & Martínková, 2018), PECs have shown high soil C sequestration rates (Chimento et al., 2016; Lemus & Lal, 2005; Sartori et al., 2006).

In addition, there are also other plant belowground organs (PBO) that significantly contribute to the high belowground C storage potential of PECs: stumps and coarse roots in woody PECs and rhizomes in herbaceous PECs. Few studies provide a complete estimate of all PEC belowground C sink components and even fewer studies provide an estimate or an assessment of the fate of the C sink after the removal of the perennial crops. After 10–20 years of cultivation, the productivity of PECs generally decreases (Arundale, Dohleman, Heaton, et al., 2014; Toenshoff, Stuelpnagel, et al., 2013) and the plantations have to be re-established or converted to arable land. Concerns exist on the fate of the soil organic C (SOC) and C of BGB biomass pools after the removal of PECs (Agostini et al., 2015; Chimento et al., 2016; Lemus & Lal, 2005).

The objective of this research was to quantify the PECs-derived belowground C and N stocks.

To achieve this, we quantified (i) the biomass and the stocks of C and N in belowground components (fine roots and plant belowground organs) and (ii) the effect of PECs cultivation on soil C and N stocks.

2 | MATERIALS AND METHODS

2.1 | Experimental field trial

The field trial was established in April 2007 in Gariga di Podenzano, Piacenza, Italy (44°58048″N, 9°41009″E). A detailed description of the experiment is provided by Amaducci et al. (2017). The soil is silt loam classified as Haplic Luvisols (Siltic, Chromic; FAO-WRB) with low carbonate content (<1%) and neutral pH (7.2). Before the establishment of the experiment, the site had hosted a cereal crop rotation (i.e. arable land), with a prevalence of maize for 30 years. The experimental layout is a completely randomized block design, with three blocks and single plot size of 600 m^2 (20 × 30 m) to compare six PECs: giant reed (*Arundo donax* L.), switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus* × *giganteus* L.), poplar (*Populus* spp.), willow (*Salix* spp.) and black locust (*Robinia pseudoacacia* spp.). Three additional plots were established in the adjacent field, where the annual crop rotation had continued.

2.2 | Soil sampling

Soil samples were collected in April 2018, 11 years after crops establishment. Six intact soil cores ($n = 3$ for soil chemical analysis and $n = 3$ for bulk density [BD]) were collected in each plot per crop treatment at a depth of 0–100 cm pressing, with the hydraulic arm of a digger, a self-constructed 'Shelby' tube sampler of 7 cm diameter (Amaducci et al., 2008). The soil cores were taken in three different positions (2 cores per position) according to the sampling strategy described by Zatta et al. (2014) to account for the different inter-row space in each species.

The coring positions were inter-row (C_i), edge of the plant (C_e) and centre of the plant (C_c). For woody species (black

locust, poplar and willow), C_i and C_e were 125 and 62.5 cm from C_c. Miscanthus and giant reed were cored at 70 (C_i) and 35 cm (C_e), while switchgrass at 20 cm (C_i) and 10 cm (C_e) from C_c. Six random soil cores were taken within the arable plots, in the adjacent field. Whole soil cores were divided in portions representative of five soil depths: 0–10; 10–20; 20–30; 30–60 and 60–100 cm (Figure 1). Soil cores for C and N content collected in the same plot were combined to have one sample per each soil depth layer.

Total number of soil samples taken from PECs plots was $n = 90$ (6 crops × 3 blocks × 5 depths) for CN analysis and $n = 270$ (6 crops × 3 positions × 3 blocks × 5 depths) for BD. Soil samples for chemical analysis were taken to the laboratory, air-dried and sieved at 2 mm. Soil samples for BD were oven dried at 105°C and weighted.

2.3 | Fine roots biomass sampling

Soil samples for fine roots biomass (FRB) were collected in April 2018 using the same approach described above for soil samples. The soil cores were taken in three different positions (Zatta et al., 2014) and divided in portions representative of six soil depths: 0–10; 10–30; 30–45; 45–60; 60–75 and 75–100 cm (Figure 1). The number of fine root samples was $n = 324$ (6 crops × 3 position × 3 blocks × 6 depths).

Before fine roots separation and analysis, soil samples were stored at −20°C. To separate fine roots from soil, samples were put in oxalic acid (2%) for 2 h, and then washed in a hydraulic sieving-centrifuge device (Monti & Zatta, 2009). Once cleaned, fine roots were hand-picked from the water using a 2 mm mesh sieve and oven dried at 65°C until constant weight. Samples were then ground and sieved at 2 mm for CN analysis.

2.4 | Stumps, coarse roots and rhizomes sampling

PBO (Stumps, coarse roots and rhizomes; Figure 1) of PECs were excavated with a digger. A different approach was applied for herbaceous and woody crops:

- for herbaceous crops, a known area to a depth of 30 cm was excavated;
- for woody crops, four pre-selected plants per plots were excavated. The selection of the plants was done according to the following steps: (1) the ratio of aboveground biomass (AGB) and BGB was assumed to be constant; (2) AGB yield was calculated with an allometric function that considers shoot diameter of plants representative of the average yield obtained in the period 2010–2018 (Amaducci et al., 2017); for this purpose, the diameter of shoots, at 130 cm from the ground, was measured with a digital caliper on 40 plants along two rows within each plot and (3) target plants were marked with spray and sampled to a depth of 30 cm since no PBO were found under this layer. Once excavated from the soil, the aboveground part of the stumps was cut and removed. Samples were washed with a high-pressure washer, to remove any soil particle, and

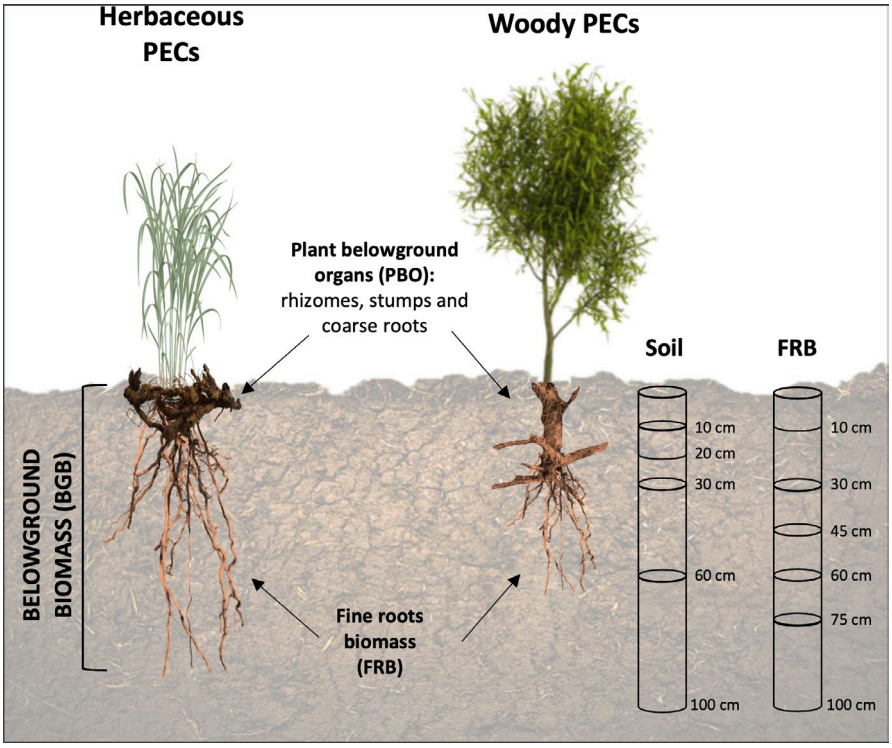


FIGURE 1 Scheme of belowground biomass components and soil layers sampled to assess the belowground carbon and nitrogen storage for herbaceous and woody perennial energy crops to 1-m depth

weighted. A sub-sample of PBO was oven dried at 65°C until constant weight, ground and sieved at 2 mm for CN analysis.

2.5 | CN analysis and stock calculation

Air-dried soil, fine roots and PBO samples were weighted and analysed by Dumas combustion method, with a CN elemental analyser, to determine C and N concentration (VarioMax CNS, Elementar, Germany). Soil BD (g cm^{-3}) was calculated dividing dry soil weight by the volume of the sample. Stones content was negligible (<0.1%). Soil organic carbon (SOC) stock (Mg C ha^{-1}) and soil total N (STN) stocks (Mg N ha^{-1}) in soil were calculated as the product of C and N concentration, BD and the width of each soil layer (m). C and N stocks in belowground biomass components were calculated as the product of dry biomass and C and N concentration in biomass in each soil layer.

The belowground C and N stocks were calculated as the sum of:

- The difference in SOC stock or STN stock between arable land and the different treatments (i.e. ΔSOC stock or ΔSTN stock);
- C and N stocks in fine roots biomass;
- C and N stock in plant belowground organs.

2.6 | Statistical analysis

Soil variables (BD, SOC content, STN content, SOC and STN stock) and PBO parameters (Biomass, C and N stocks) were analysed using a one-way mixed-model ANOVA for complete randomized block design. Crop type was considered as fixed main effects with block as a random effect. Mixed model was run independently for each soil layer. Means were compared using Tukey's honestly significant difference ($\alpha = 0.05$). Log transformations were performed to satisfy assumptions of normality and heteroskedasticity when needed. Fine roots parameters (Biomass, C and N stocks) and belowground C and N stocks were analysed with a two-way mixed-model ANOVA for complete randomized block design. Crop type and soil depth and their interaction were considered as fixed main effects with block as a random effect. Analyses were performed using R: nmle (Pinheiro et al., 2020) and multcomp (Hothorn et al., 2008) packages.

Linear regression was used to detect the influence of fine roots C stock on soil ΔSOC (g kg^{-1}) at different soil depth layers. All statistical analyses were performed with R 3.6.2.

3 | RESULTS

3.1 | Belowground biomass

3.1.1 | Plant belowground organs

PBO biomass was significantly affected by PEC species ($p < 0.001$; Figure 2) and had different C ($p < 0.05$) and N ($p < 0.001$) concentrations among PECs (Tables S2 and S3).

PBO biomass, as an average of all PECs, was 31.9 Mg DM ha^{-1}, with giant reed having the highest value (50.7 Mg DM ha^{-1}) and switchgrass the lowest (5.9 Mg DM ha^{-1}; Figure 2a). C content in PBO biomass was similar for all PECs (44%) apart from switchgrass, which had the lowest value of C content in PBO (40%; Table S3). This small difference in C content did not change the ranking of PECs for PBO C stocks (Figure 2b), which is the same described for PBO biomass. N concentration in PBO was highest in black locust (1.9%), and similar among all other PECs (0.7%; Table S1). Black locust had the highest N stock

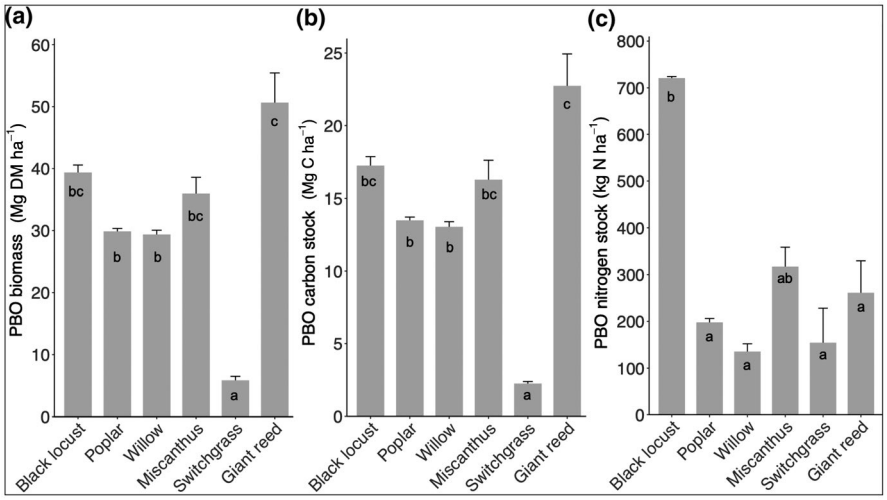


FIGURE 2 Plant belowground organs (PBO) dry biomass (a), carbon stock (b) and nitrogen stock (c) in biomass per hectare in the 0–30 cm of soil of each perennial energy crops after 11 years of cultivation. Different letters indicate significant differences ($p < 0.05$) between crops according to Tukey's test

inn PBO (720 kg N ha^{-1}) and switchgrass the lowest one (154 kg N ha^{-1}; Figure 2c).

3.1.2 | Fine roots biomass

Fine roots biomass (FRB) was significantly different among PECs ($p < 0.001$) and at different depth layers ($p < 0.001$; Table S2). A significant interaction was found between the two factors ($p < 0.002$; Table S2). In the 0–30 cm layer (topsoil), switchgrass and miscanthus had higher FRB than giant reed and all woody crops (Figure 3a). In the 30–100 cm layer (subsoil), switchgrass had the highest FRB (Figure 3a). C and N concentrations in FRB were significantly different among PECs ($p < 0.001$; Table S2). Black locust had the highest N concentration in FRB (2.1% on average, Table S3), whereas switchgrass and giant reed the lowest.

3.2 | Effect of PECs on soil

BD, SOC content and STN content, SOC stock and STN stock differed significantly, after 11 years of cultivation, between PECs and arable land as a function of the soil layers (Table 1).

PECs had lower values of BD in the 0–10 cm layer than arable land (Table 1). On average, the reduction of BD under PECs in the 0–10 cm layer was of 0.15 g cm^{-3} (−10%). Herbaceous PECs had higher values of BD in the 10–20 cm soil layer than arable land (Table 1). The increment of BD in the 10–20 cm layer under herbaceous PECs was on average 0.10 g cm^{-3} (+8%). Giant reed was the only PEC to affect BD in the 20–30 cm layer compared to arable land, increasing the BD by 0.25 g cm^{-3} (+8.5%; Table 1).

In the 0–10 cm layer, PECs increased SOC content and SOC stock compared to arable land, respectively, by +76% (+5.5 g kg^{-1}) and +49% (6.3 Mg C ha^{-1}; Table 1). In the 0–10 cm layer, poplar had the highest increment of SOC content and willow had the highest increment of SOC stock while switchgrass had the lowest increment for both SOC content and SOC stock (Table 1). All PECs, with the exception of switchgrass, increased significantly SOC stock in the 0–30 cm (Table 1) with respect to the reference arable land (+5.42 Mg C ha^{-1} on average).

PECs significantly increased STN content and STN stock by comparison to arable land in the 0–10 and 30–60 cm depth layers (Table 1). PECs had higher average values of STN content (+49%, +0.47 g kg^{-1}) and higher STN stock than arable land in the 0–10 cm depth layer, while black locust, miscanthus and giant reed also had significantly higher STN content (+26%, 0.16 g kg^{-1} on average) than arable land in the 30–60 cm depth layer, as well as higher STN stock values (+28%, +0.75 Mg N ha^{-1} on average). Considering the 0–30 cm depth layer, miscanthus and giant reed had higher STN stock than arable land (Table 1).

3.3 | Belowground C and N stocks of PECs

Belowground C stock (ΔSOC stock + belowground biomass C stock) differed significantly among PECs ($p < 0.005$) and depth layers ($p < 0.001$) and a significant interaction was found between the two factors ($p < 0.001$; Table S2).

The belowground C stock in the topsoil showed significant differences only between giant reed (32.9 Mg C ha^{-1}) and switchgrass (14.6 Mg C ha^{-1}; Figure 4a). In the subsoil, switchgrass had a higher belowground C stock than black locust (Figure 4a). Giant reed, miscanthus, willow, poplar and black locust had a higher belowground C stock in the topsoil than in the subsoil (Figure 4a). On average, 43% of the belowground C stock of PECs was allocated in the PBO (Figure 6). Woody PECs had higher proportion of belowground C stock allocated in PBO (60%) than herbaceous crops (38%). On average, the proportion of belowground C stock accounted for by fine roots was 20%. The percentage of belowground C stock accounted for by fine roots was higher for herbaceous crops (28%) than woody crops (11%). Among herbaceous crops, switchgrass had the highest proportion of belowground C stock in fine roots (55%).

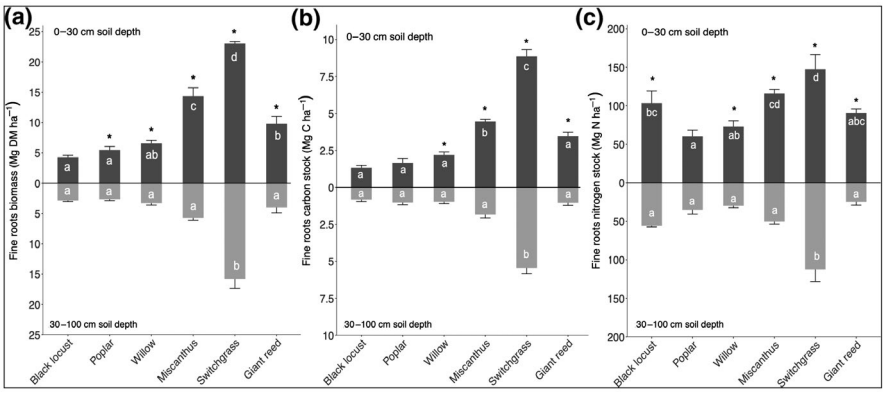


FIGURE 3 Fine roots biomass (a), carbon stock (b) and nitrogen stock (c) in biomass per hectare of each perennial energy crops after 11 years of cultivation between 0–30 and 30–100 cm of soil for each species. Different letters indicate significant differences ($p < 0.05$) between crops according to Tukey's test for each depth. * indicates significant differences between depths of the same crops according to Tukey's test

TABLE 1 Soil bulk density (BD, g cm^{-3}), soil organic carbon content (SOC content, g kg^{-1}), SOC stock (Mg C ha^{-1}), soil total nitrogen content (STN content, g kg^{-1}) and STN stock (Mg N ha^{-1}) at different soil depth under six PECs after 11 years from the establishment

Soil depth (cm)	Woody PECs			Herbaceous PECs			Arable	*F*	*p*
	Black locust	Poplar	Willow	Miscanthus	Switchgrass	Giant reed			
BD									
0–10	1.28 ± 0.02 a	1.27 ± 0.01 a	1.31 ± 0.02 a	1.32 ± 0.02 ab	1.33 ± 0.02 ab	1.28 ± 0.05 a	1.45 ± 0.04 b	3.98	0.020
10–20	1.42 ± 0.01 a	1.39 ± 0.01 a	1.37 ± 0.01 a	1.52 ± 0.01 b	1.54 ± 0.02 b	1.55 ± 0.01 b	1.43 ± 0.01 a	22.95	<0.001
20–30	1.45 ± 0.02 ab	1.41 ± 0.01 a	1.40 ± 0.01 a	1.41 ± 0.04 a	1.43 ± 0.01 a	1.53 ± 0.01 b	1.41 ± 0.02 a	5.25	0.007
30–60	1.50 ± 0.01	1.44 ± 0.02	1.46 ± 0.01	1.43 ± 0.03	1.45 ± 0.02	1.52 ± 0.07	1.46 ± 0.04	0.82	0.575
60–100	1.65 ± 0.01	1.65 ± 0.02	1.68 ± 0.01	1.68 ± 0.02	1.67 ± 0.01	1.67 ± 0.01	1.67 ± 0.01	2.08	0.132
SOC content									
0–10	13.50 ± 0.41 c	14.04 ± 0.62 c	13.81 ± 0.29 c	12.88 ± 0.26 c	10.11 ± 0.27 b	12.68 ± 0.33 c	7.29 ± 0.31 a	39.55	<0.001
10–20	7.80 ± 0.26 b	6.61 ± 0.07 a	7.42 ± 0.25 ab	7.33 ± 0.15 ab	7.60 ± 0.30 ab	7.58 ± 0.26 ab	7.51 ± 0.08 ab	3.12	0.044
20–30	7.42 ± 0.07 b	6.15 ± 0.09 a	7.14 ± 0.58 ab	6.90 ± 0.19 ab	7.35 ± 0.10 ab	7.04 ± 0.19 ab	7.73 ± 0.09 b	3.84	0.022
30–60	5.13 ± 0.01 abc	5.26 ± 0.18 abc	4.83 ± 0.05 ab	6.04 ± 0.31 c	5.91 ± 0.29 bc	5.50 ± 0.27 abc	4.70 ± 0.06 a	5.41	0.006
60–100	3.03 ± 0.04	3.45 ± 0.09	3.55 ± 0.20	3.10 ± 0.12	3.50 ± 0.26	3.67 ± 0.66	3.24 ± 0.08	0.88	0.537
SOC stock									
0–10	17.28 ± 0.52 c	17.84 ± 0.79 c	18.03 ± 0.38 c	17.01 ± 0.34 c	13.41 ± 0.35 b	16.24 ± 0.43 c	10.52 ± 0.23 a	32.16	<0.001
10–20	11.04 ± 0.37 bc	9.20 ± 0.09 a	10.17 ± 0.34 ab	11.13 ± 0.23 bc	11.70 ± 0.45 bc	11.75 ± 0.40 c	10.74 ± 0.10 abc	8.16	0.001
20–30	10.72 ± 0.11 b	8.70 ± 0.13 a	9.99 ± 0.82 ab	9.71 ± 0.27 ab	10.52 ± 0.14 ab	10.78 ± 0.29 b	10.86 ± 0.17 b	4.41	0.014
30–60	23.11 ± 0.04 ab	22.77 ± 0.77 ab	21.14 ± 0.22 ab	25.82 ± 1.32 b	25.73 ± 1.28 b	25.10 ± 1.24 ab	20.98 ± 0.06 a	4.72	0.011
60–100	20.00 ± 0.38	22.80 ± 0.79	23.88 ± 1.32	20.82 ± 1.06	23.41 ± 1.84	24.55 ± 4.52	21.66 ± 0.60	0.96	0.4877
0–30	39.04 ± 1.62 b	35.74 ± 1.59 ab	38.19 ± 2.16 b	37.85 ± 0.86 b	35.62 ± 0.38 ab	38 .76 ± 1.06 b	32.11 ± 0.52 a	7.75	0.001
30–100	43.10 ± 0.71	45.56 ± 2.41	45.02 ± 1.91	46.64 ± 2.17	49.14 ± 4.67	49.65 ± 1.62	42.64 ± 1.07	2.53	0.08
STN content									
0–10	1.38 ± 0.04 b	1.50 ± 0.01 b	1.50 ± 0.04 b	1.39 ± 0.06 b	1.27 ± 0.06 b	1.45 ± 0.04 b	0.95 ± 0.03 a	14.44	<0.001
10–20	0.92 ± 0.04	1.01 ± 0.06	0.93 ± 0.03	0.91 ± 0.05	0.96 ± 0.04	0.96 ± 0.04	0.98 ± 0.01	0.89	0.530
20–30	0.90 ± 0.02	0.72 ± 0.07	0.91 ± 0.06	0.95 ± 0.07	0.92 ± 0.02	0.98 ± 0.08	1.01 ± 0.01	2.59	0.076
30–60	0.77 ± 0.02 b	0.71 ± 0.01 ab	0.66 ± 0.01 ab	0.79 ± 0.05 b	0.73 ± 0.01 ab	0.74 ± 0.04 b	0.61 ± 0.01 a	5.70	0.005
60–100	0.51 ± 0.01	0.50 ± 0.01	0.51 ± 0.01	0.50 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.50 ± 0.01	0.97	0.486
STN stock									
0–10	1.77 ± 0.05 b	1.91 ± 0.01 b	1.96 ± 0.05 b	1.83 ± 0.09 b	1.69 ± 0.08 b	1.86 ± 0.06 b	1.29 ± 0.03 a	11.52	<0.001
10–20	1.31 ± 0.06	1.41 ± 0.08	1.27 ± 0.04	1.39 ± 0.07	1.48 ± 0.06	1.49 ± 0.06	1.27 ± 0.01	3.18	0.058

(Continues)

TABLE 1 (Continued)

Soil depth (cm)	Woody PECs			Herbaceous PECs			Arable	*F*	*p*
	Black locust	Poplar	Willow	Miscanthus	Switchgrass	Giant reed			
20–30	1.30 ± 0.03 ab	1.02 ± 0.10 a	1.28 ± 0.09 ab	1.34 ± 0.10 ab	1.32 ± 0.02 ab	1.50 ± 0.13 b	1.25 ± 0.02 ab	2.84	0.041
30–60	3.47 ± 0.08 b	3.09 ± 0.02 ab	2.88 ± 0.02 ab	3.36 ± 0.21 b	3.16 ± 0.05 ab	3.39 ± 0.19 b	2.66 ± 0.10 a	5.45	0.006
60–100	3.35 ± 0.01	3.32 ± 0.04	3.42 ± 0.01	3.38 ± 0.05	3.38 ± 0.01	3.39 ± 0.01	3.35 ± 0.03	1.92	0.158
0–30	4.37 ± 0.13 ab	4.33 ± 0.04 ab	4.51 ± 0.28 ab	4.55 ± 0.40 b	4.49 ± 0.20 ab	4.86 ± 0.36 b	3.81 ± 0.04 a	4.51	0.012
30–100	6.81 ± 0.13 b	6.41 ± 0.10 ab	6.30 ± 0.05 ab	6.74 ± 0.30 b	6.53 ± 0.08 ab	6.77 ± 0.32 b	6.00 ± 0.13 a	6.35	0.003

Note: Values represent mean ± standard error ($n = 3$). Different letters within the same row indicate significant differences ($p < 0.05$) among crops within soil layer, according to Tukey's test.

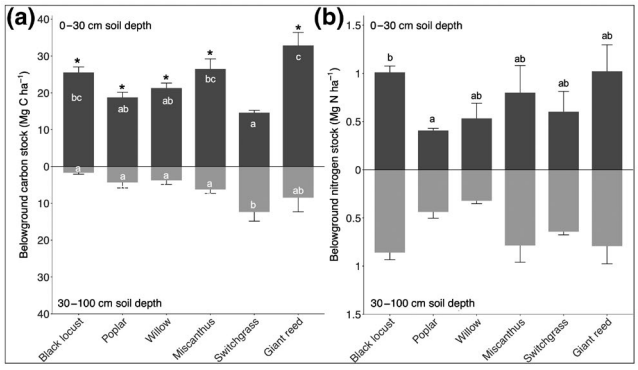


FIGURE 4 Belowground carbon stock (a), belowground nitrogen stock (b) per hectare of each perennial energy crops after 11 years of cultivation between 0–30 and 30–100 cm of soil for each species. Different letters indicate significant differences ($p < 0.05$) between crops according to Tukey's test for each depth. * indicates significant differences between depths of the same crops according to Tukey's test

Belowground N stock differed significantly among PECs ($p < 0.02$) but no depth effect was found (Table S2; Figure 4b).

4 | DISCUSSION

4.1 | Belowground biomass of PECs

To our knowledge, this is the first study where belowground biomass (considering rhizomes and stumps from fine roots biomass separately) of six different PECs was measured in the same long-term (>10 years) field experiment. Very few studies report data on BGB of single PECs, even though many report data on FRB. Research on belowground plants compartments (fine roots vs. stumps and rhizomes) to improve our understanding on C allocation dynamics, and the effect of their chemical composition, longevity, turnover and decomposition rates on soil C dynamics (Klimešová & Martínková, 2018). This is particularly relevant for perennial crops, where BGB may persist in the soil for many years (Nobis & Schweingruber, 2013). Data on plant belowground organs (PBO), found in this research for herbaceous crops, are in line with those found in similar pedoclimatic conditions for miscanthus (Richter et al., 2015) and switchgrass (Wilson et al., 2013) in crop stands older than 6 years while no data are available for giant reed old stands (Roncucci et al., 2012).

Results on PBO characterization of woody crops are in line with those reported by Johansson and Hjelm (2012) and Quinkenstein et al. (2012) for SRC-poplar and black locust plantations of the same age. Regarding SRC-willow no studies on long-term plantations were found.

Characterization of fine roots system for the six PECs of this study had been already performed in the same experiment

after 6 years from planting (Chimento & Amaducci, 2015) and only for the herbaceous species in a similar environment (Monti & Zatta, 2009). Ranking of PECS for fine root biomass in this study was the same found by Chimento and Amaducci (2015): switchgrass > miscanthus > giant reed > willow > poplar > black locust. Despite this similarity, we found that in the last 5 years, the FRB has increased for all PECs. The increase in the FRB, at least for herbaceous PECs, is similar to that reported for a younger plantation by Kibet et al. (2015), but higher than that reported for plantations of asimilar age (Christensen et al., 2016; Kahle et al., 2001; Richter et al., 2015). On the contrary, our results on FRB of woody PECs older than 6 years are similar to those reported by Al Afas et al. (2008) and Quinkenstein et al. (2012). The spatial distribution and development of BGB are different for herbaceous and woody PECs: in herbaceous PECs, fine roots system develop from rhizome, which have the capacity of lateral spreading, creating multiple rooting units (Klimešová & Martínková, 2018). In tree plants, as well as in woody PECs, stumps and coarse roots, from which the other fine roots orders generate, increase their biomass during the lifetime of the crop but do not have the same spreading capacity of hearbacous PECs (Klimešová & Martínková, 2018; Figure S3). For this, woody PECs might have peaked their fine roots production within the first 6 years from planting while herbaceous PECs require more time. In addition, woody PECs fine roots were mainly found in the first 30 cm of soil while herbaceous crops tended to distribute a large proprortion of fine roots at deeper soil layers (Figure S1).

4.2 | Effect of PECs on soil

Soil bulk density, soil organic carbon content, soil total nitrogen content, soil organic carbon stock and soil total nitrogen stocks were all affected by PECs after 11 years of cultivation.

Under all PECs, BD of the first soil layer (0–10 cm) was lower than that found in the same experiment by Chimento et al. (2016) than that of the arable land, whereas BD increased in the 10–20 cm depth layer under herbaceous PECs and, in the 20–30 cm layer, it was higher under giant reed than arable land. The increase in BD during PEC cultivation is a consequence of the limited soil disturbance (Lal, 2011); in fact, other authors reported an increase in BD in the first years after the establishment of PECs (Hu et al., 2018; Lai et al., 2018; Medinski et al., 2014; Zimmermann et al., 2013). In our experiment, after 11 years of PECs, the increase in soil C (Ruehlmann & Körschens, 2009) and the effect of root growth (Douglas et al., 1992; Rasse et al., 2000; Tolbert et al., 2000) seem to have progressively reduced soil compaction and increased soil porosity (Lal, 2011). A positive linear correlation (though not significant) between ΔSOC content and soil porosity was found in the 0–10 cm depth layer (Figure S1). At deeper soil layers, where no increments in soil C occurred, no correlation was found between ΔSOC content and porosity (Figure S1). Other authors have reported a reduction of soil compaction in PEC experimental trials older than 6 years (Holder et al., 2019; Matos et al., 2012; Walter et al., 2015; Zatta et al., 2014), as well as an increase in BD in the 10–20 cm depth layer under herbaceous rather than to woody PECs and arable land in long-term PEC experimental trials (Ferchaud et al., 2016; Holder et al., 2019; Zatta et al., 2014). Ferchaud et al. (2016), in particular, hypothesized that the highest soil compaction under herbaceous PECs was a consequence of harvesting operations. Differences in harvesting operations in this study between woody and herbaceous crops are significant: woody crops were manually harvested every 2 years; herbaceous crops were harvested every year with normal farm machines with four mechanical operations (cutting, mulching, windrowing and bailing). This could have particularly affected giant reed, compacting the soil in the 20–30 cm depth layer because harvest operations of PECs occur in late winter, when soil conditions can favour compaction.

After 11 years of cultivation, SOC content was higher under PECs than arable land in the 0–10 cm depth layer. SOC content kept increasing in the 0–10 cm depth layer from the 6th to the 11th year from establishment as indicated by the comparison of our results to the ones reported by Chimento et al. (2016). In the top layer, woody crops showed higher SOC content increments than herbaceous crops (Chimento et al., 2016; Rowe et al., 2016). In general, SOC content in the 10–20 and 20–30 cm depth layers showed lower values after 11 years than after 6 years for all PECs. Large C input from FRB under PECs have usually been associated with the potential of high SOC sequestration rates especially at deep soil layers where conditions are less favourable for microbial decomposition (de Graaff et al., 2014). On the contrary, in upper soil layers C input from fine roots can trigger fast metabolic processes by soil microorganisms, thus accelerating SOC turnover, namely the 'priming effect' (Kuzyakov, 2002; Kuzyakov et al., 2000; Lange et al., 2015; Zatta et al., 2014). We analysed the correlation between fine roots C stock and ΔSOC using data from Chimento and Amaducci (2015), Chimento et al. (2016) and this study, at three soil depths (0–10, 10–30 and 30–100 cm): no significant correlation between C stock of fine roots and ΔSOC content was found in the 0–10 cm depth layer after 6 years (Figure 5), suggesting that C sequestration of PECs in the first years of cultivation is not caused by fine roots but by other types of C sources, such as litter fall or harvest residues. For example, PECs without litter fall C input (i.e. switchgrass and giant reed), both after 6 and 11 years, sequestered less C compared to woody crops and miscanthus, which are charactherized by litter fall C input (Amougou et al., 2012; Boman & Turnbull, 1997). Likewise, a significant negative correlation between fine roots C stock and ΔSOC content was found in the 0–10 cm depth layer after 11 years (Figure 5). Switchgrass, which had the highest fine roots C stock, had the

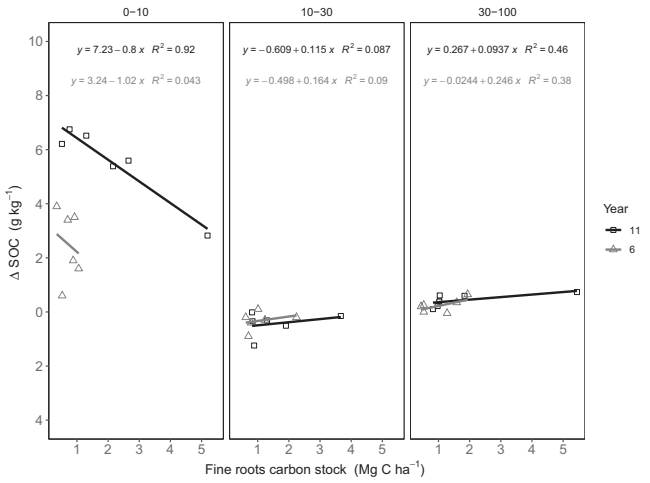


FIGURE 5 Correlation between fine roots carbon stock and ΔSOC between years 0–6 and 0–11 of three soil depths (0–10, 10–30 and 30–100 cm) of six perennial energy crops grown on former arable marginal land in Gariga, Italy. Data for fine roots 0–6 taken from Chimento and Amaducci (2015) and ΔSOC 0–6 calculated from data of Chimento et al. (2016) in the same field trial after 6 years from the establishment. Colours of equation and R^2 are black for 11 years and grey for 6 years

lowest C sequestration in the first soil layer. In the 10–30 cm depth layer after 6 and 11 years, the fine roots C stock is not correlated with SOC content (Figure 5), suggesting that the high C stock of fine roots may have induced the priming effect. Easily-decomposable plant C inputs from deep-rooting system of PECs positively impact belowground functioning and microbial metabolism (Ferrarini et al., 2020), resulting in higher microbial turnover in the rhizosphere (Kuzyakov, 2002; Kuzyakov et al., 2000). At deeper soil layers (30–100 cm), a positive, but not significant correlation was found between SOC contentand fine roots C stock (Figure 5). At this depth, chemical C stabilization of recalcitrant material became prevalent over enzymatic decomposition (de Graaff et al., 2014; Fontaine et al., 2007; Rumpel & Kögel-Knabner, 2011; Schnecker et al., 2015).

PECs had significantly higher SOC stock in the 0–30 cm layer (with the exception of switchgrass) than arable land (Table 1). Annual SOC sequestration rates in the 0–10 cm layer under woody PECs and miscanthus were higher than switchgrass and giant reed, which is in agreement with previous research (Ceotto & Di Candilo, 2011; Chimento et al., 2016). The lower C sequestration rates of herbaceous than woody crops after 11 years is a consequence of the effect of different factors such as C input dynamics from litter and roots, as explained above (Lange et al., 2015; Poeplau & Don, 2014; Zatta et al., 2014; Zimmermann et al., 2013). SOC sequestration rates in our experiment of giant reed, poplar and willow in the 0–10 cm layer (Table 2) are in agreement with previous research (Hellebrand et al., 2010; Monti & Zegada-Lizarazu, 2016), while for miscanthus, switchgrass and black

TABLE 2 SOC sequestration rate (Mg C ha^{-1} $year^{-1}$) of six PECs at different depth layer after 11 years from establishment

	Crop	SOC sequestration rate (Mg C ha^{-1} $year^{-1}$)		
	Layer	**0–10 cm**	**10–30 cm**	**0–30 cm**
Woody PECs	Black locust	0.61	0.01	0.62
	Poplar	0.67	−0.34	0.33
	Willow	0.68	−0.13	0.55
Herbaceous PECs	Miscanthus	0.59	−0.07	0.52
	Switchgrass	0.26	0.06	0.32
	Giant reed	0.52	0.08	0.60

locust in particular they were lower than that reported by several authors in different environments (Anderson-Teixeira et al., 2009; Bandaru et al., 2013; Cattaneo et al., 2014; Matos et al., 2012; Nocentini et al., 2015; Quinkenstein & Jochheim, 2016). The differences among these data depend on differences in climates, soil types, initial SOC levels (Chimento et al., 2016; Dondini et al., 2009) and harvesting approach: for example, in our case, the mechanical harvest of herbaceous crops, especially windrowing and bailing operation, might have partially removed the litter input of miscanthus into soil, leading to a lower SOC sequestration rate in upper soil layers. SOC sequestration rates in the 10–30 cm (Table 2) layer suggest that the effect of C input from PECs did not increase soil C, though the priming effect on SOC may have occurred, as explained above, especially under woody crops (Walter et al., 2015) and miscanthus (Zatta et al., 2014; Table 2). In the case of black locust, the lower C/N ratio of the input with respect to the other PECs may have reduced the priming effect (Zang et al., 2016). Considering the 0–30 cm layer, black locust had the highest SOC sequestration rate among PECs, whereas switchgrass and poplar had the lowest. The SOC sequestration rates in the 0–30 cm layer of PECs, that is on average 0.49 Mg C ha^{-1} $year^{-1}$ (Table 2), are in agreement with previous research (Bandaru et al., 2013; Cattaneo et al., 2014; Dufossé et al., 2014; Monti & Zegada-Lizarazu, 2016; Quinkenstein & Jochheim, 2016; Rytter et al., 2015).

The increase in STN content in the 0–10 cm layer is a consequence of the increase in SOC content in the same soil layer (Ferrarini et al., 2020; Kahle et al., 2013) and of the accumulation of N from litter (Cadoux et al., 2012). The increase in STN content in the 30–60 soil layer is probabily a consequence of N leached from upper soil layers, as was found in other studies on PECs (Devine et al., 2004; Dufossé et al., 2014; Kahle et al., 2013).

4.3 | Belowground C and N stocks

In this work, for the first time, a comparison of belowground C and N stocks and their components is presented

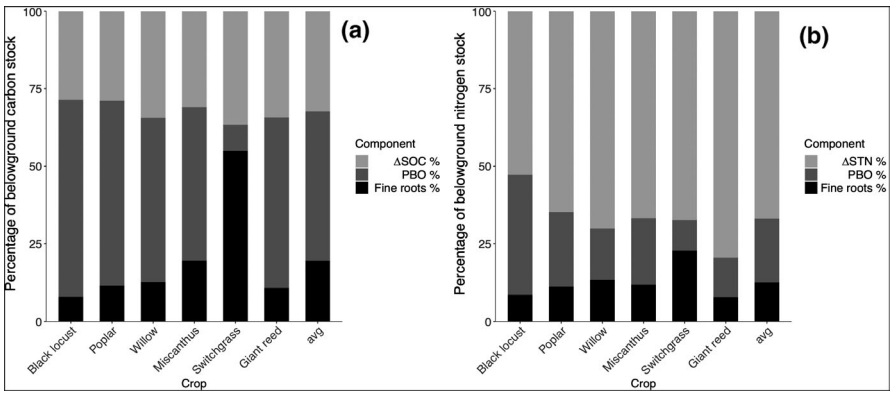


FIGURE 6 Relative contribution (%) to belowground carbon stock (a) and belowground nitrogen stock (b) of soil, plant belowground organs and fine roots biomass

for six PECs grown in the same experimental condition for 11 years. When cultivated on marginal lands, the six PECs involved in this study have been already recognized as valuable crops for their environmental (Chimento et al., 2016; Chimento & Amaducci, 2015) and productive benefits (Amaducci et al., 2017). An accurate assessment of their C and N stocks not only in soil, but in BGB was necessary to depict the overall contribution of each component to belowground C and N storage at system level. On average, 68% of the belowground C stock of PECs was found in BGB and 32% in soil (Figure 6): after 11 years of PEC cultivation, the C stocked in the BGB was twice as large as the one stocked in the soil. In addition, 79% of belowground C stock of PECs was found in the 0–30 cm depth layer and only 21% in the 30–100 cm depth layer (Figure S4). Switchgrass had the highest proportion of its belowground C (46%) stored in the subsoil (30–100 cm) while black locust had the lowest (6%; Figure S4). This is a crucial aspect in the study of these crops, not only during their life-time period but also considering potential benefits at the end of their plantation and after re-conversion to arable land. In these terms, giant reed showed the highest belowground C stock in the 0–30 cm depth layer (i.e. potentially re-convertible soil layer) while switchgrass showed the lowest. Herbaceous crops, switchgrass in particular, had the highest potential of C storage in the subsoil (Figure S4). Some authors have argued that PECs able to allocate high amount of C in deeper soil layers are preferable because this amounts of C would not be affected by future operations (Chimento & Amaducci, 2015; de Graaff et al., 2014). There are concerns that at the end of the PEC cultivation cycle, the conversion of these crops back to arable land could negatively affect the SOC stock. However, a positive effect of conversion on SOC is possible, due to the high amount of crop residues entering to the soil as new particulate organic matter (POM) with the disruption of BGB during the re-conversion process (Toenshoff, Georg, et al., 2013; Toenshoff, Stuelpnagel, et al., 2013; Wachendorf et al., 2017).

Belowground N stock of PECs showed a net storage of N of 1.35 Mg N ha^{-1} on average, with a rate of N accumulation of 122 kg N ha^{-1} $year^{-1}$ that is quite high, considering that the only fertilizers applied to the experimental trial were 100 kg of N for willow and poplar after harvest (400 kg N ha in 11 years). These high rates of N accumulation can only be explained assuming a relevant N fixation process for PECs (Dohleman et al., 2012). The hypothesis of N fixation for PECs is a well-known fact for crops belonging to Leguminosae family such as black locust (Danso et al., 1995; Marron et al., 2018), but it has recently been confirmed to also occur in miscanthus (Cadoux et al., 2012; Davis et al., 2010; Eckert et al., 2001; Jørgensen, 2011; Miyamoto et al., 2004; Smith & Slater, 2010), switchgrass (Bahulikar et al., 2014; Kämpfer et al., 2015; Kim et al., 2012; Roley et al., 2018, 2019; Xu et al., 2018), giant reed (Smith & Slater, 2010; Xu et al., 2018), willow (Pugesgaard et al., 2015; Rooney et al., 2009; von Wuehlisch, 2011) and poplar (Doty et al., 2009, 2016; Rooney et al., 2009; von Wuehlisch, 2011). Based on N balance calculation of input and output (data not shown), it is reasonable to assume that N fixation accounted for the excess of N under PECs in our experiment. We calculated a rate of N fixation of 200 kg N ha^{-1} $year^{-1}$ for black locust, that is consistent with the results of Danso et al. (1995) and Marron et al. (2018); 30 kg N ha^{-1} $year^{-1}$ for poplar and willow, 120 kg N ha^{-1} $year^{-1}$ for miscanthus that is in the range of N fixation rates calculated by Davis et al. (2010) with the DAYCENT model; 80 kg N ha^{-1} $year^{-1}$ for switchgrass that is higher than the value of 50 kg N ha^{-1} $year^{-1}$ measured by Roley et al. (2019) or 60 kg N ha^{-1} $year^{-1}$ by Roley et al. (2018) and 160 kg N ha^{-1} $year^{-1}$ for giant reed.

Further studies are needed to consolidate the database relative to the BGB of PECs. This is highly relevant when accounting for the overall biogenic C sequestration potential of these perennial crops in LCA studies but also in modelling scenario studies to predict the legacy of PECs on the C cycle when plantations are terminated and the land is converted to arable crops.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: A.F. and S.A. Performed the experiments: E.M., A.F., P.S., A.M. and M.P. Performed the laboratory soil analysis: E.M. and A.M. Analysed the data: E.M. and A.F. Contributed reagents/materials: S.A. Wrote the paper: E.M., F.A. and S.A.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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