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Early impacts of marginal land-use transition to Miscanthus on soil quality and soil carbon storage across Europe

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Title: Early impacts of marginal land-use transition to *Miscanthus* on soil quality and soil C storage across Europe

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SUPPLEMENTARY MATERIAL

Rhizome biomass estimation

Allometric equations are widely used for plant biomass assessment. Belowground plant biomass can be estimated based on allometric relations from aboveground biomass which can be measured and sampled more easily in the field.

In this work, an allometric equations was developed to estimate rhizome biomass (RB) of *Miscanthus*. Data on both above- and belowground biomass component of this crop were collected from 10 published research on *Miscanthus x giganteus* where aboveground biomass (AGB) and RB were measured at harvest time in late winter. The list of references collected is reported in Table S1 where 34 observations for AGB and RB and site information as well are reported. From each reference data on RB, AGB at RB sampling year, cumulative AGB (CumAGB), ratio between RB/CumAGB, textural class, site location, weather conditions (MAT and MAP) and years after establishment (YAE) were extracted or calculated when necessary. We averaged the RB and cumulative AGB data per years after establishment (Tab. S2) and we identified a significant allometric relationship based on a logarithmic regression function (Fig. S1a) between these two variables. The equation is valid for cumulative AGB values higher than 3.7963 Mg DM ha⁻¹. This formula was used to estimate RB at 4th growing season in this study. RB for this study was calculated using cumulative AGB data for each genotype/replicate reported by Awty-Carrol et al. (2022). Interestingly we also identified a significant relationship between years after establishment and the RB/CumAGB ratio (Fig. S1b).

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Table S1. Dataset collected from published literature to derive allometric equation to estimate

Miscanthus RB

Reference	Country	Sand	Silt	Clay	Soil textural class	MAT	MAP	YAE	AGB *	Cum_AGB**	RB	RB/CumAGB ratio
		%	%	%		°C	mm	years	Mg DM ha ⁻¹	Mg DM ha ⁻¹	Mg DM ha ⁻¹	
Himken et al 1997	Germany	80.4	14.8	4.8	Loamy Sand	9.3	715	4	18.1	51.1	15.1	0.30
Himken et al 1997	Germany	80.4	14.8	4.8	Loamy Sand	9.3	715	4	17.5	50.5	17.2	0.34
Himken et al 1997	Germany	80.4	14.8	4.8	Loamy Sand	9.3	715	4	15.8	48.8	15.2	0.31
Carvalho et al 2017	USA	15	70	15	Silt Loam	11.01	1042	1	1.0	1.0	0.3	0.34
Carvalho et al 2017	USA	15	70	15	Silt Loam	11.01	1042	2	3.5	4.5	1.5	0.33
Amougou et al 2011	France	7.8	72.2	19.9	Silt Loam	11.1	713	2	14.3	20.3	12.8	0.63
Amougou et al 2011	France	7.8	72.2	19.9	Silt Loam	11.1	713	2	15.3	35.6	10.2	0.29
Strullu et al. 2011	France	5	74	19	Silt Loam	10.7	625	3	20.0	33.0	16.0	0.48
Carvalho et al 2017	USA	15	70	15	Silt Loam	11.01	1042	3	11.6	44.6	4.2	0.09
Amougou et al 2011	France	7.8	72.2	19.9	Silt Loam	11.1	713	3	23.5	30.4	15.4	0.51
Amougou et al 2011	France	7.8	72.2	19.9	Silt Loam	11.1	713	3	26.1	56.5	17.3	0.31
Anderson-Teixeira et al 2013	USA	15	70	15	Silt Loam	11.01	1042	4	17.5	48.2	11.3	0.23
Strullu et al. 2011	France	5	74	19	Silt Loam	10.7	625	4	22.0	70.2	20.0	0.29
Carvalho et al 2017	USA	15	70	15	Silt Loam	11.01	1042	4	14.3	84.5	6.0	0.07
Dohleman et al. 2012	USA	10	60	30	Silty Clay Loam	12.2	962	5	44.1	129.7	23.8	0.18
Dohleman et al. 2012	USA	10	60	30	Silty Clay Loam	12.1	862	6	38.2	167.9	23.8	0.14
Dohleman et al. 2012	USA	10	60	30	Silty Clay Loam	10.2	1336	7	36.3	204.2	23.8	0.12
Kahle et al. 2001	Germany	62.1	25.4	10.4	Sandy Loam	7.9	547	4	7.5	20.8	7.7	0.37
Kahle et al. 2001	Germany	62.1	25.4	10.4	Sandy Loam	7.9	547	5	11.1	31.9	11.4	0.36
Kahle et al. 2001	Germany	62.1	25.4	10.4	Sandy Loam	7.9	547	6	12.6	44.5	12.5	0.28
Kahle et al. 2001	Germany	65.3	31	3.1	Sandy Loam	8.8	600	5	13.5	36.5	16.3	0.45
Kahle et al. 2001	Germany	65.3	31	3.1	Sandy Loam	8.8	600	6	8.8	45.3	14.6	0.32
Kahle et al. 2001	Germany	65.3	31	3.1	Sandy Loam	8.8	600	7	11.5	56.8	16.9	0.30
Kahle et al. 2001	Germany	18.6	57.6	24	Silt Loam	9.5	603	6	16.4	52.6	17.4	0.33
Kahle et al. 2001	Germany	18.6	57.6	24	Silt Loam	9.5	603	7	16.8	69.4	18.1	0.26
Kahle et al. 2001	Germany	18.6	57.6	24	Silt Loam	9.5	603	8	19.8	89.2	20.3	0.23
Himken et al. 1997	Germany	80	15	5	Sandy	9.3	715	4	16	49	16	0.33
Himken et al. 1997	Germany	80	15	5	Sandy	9.3	715	4	15	48	16	0.33
Himken et al. 1997	Germany	80	15	5	Sandy	9.3	715	4	15	48	17	0.35
Chstrian et al.2006	UK	27	50	23	Clay loam	8	600	1	6.99	6.99	4.2	0.60
Chstrian et al.2006	UK	27	50	23	Clay loam	8	600	2	10.07	17.06	6.71	0.39
Chstrian et al.2006	UK	77		23	Clay loam	8	600	3	14.03	31.09	10.23	0.33
Clifton-Brown et al. 2007	Ireland	85	10	5	Sandy Loam	9.9	873	12	10	124	20.5	0.17
Martani et al 2020	Italy	8	70	22	Silt Loam	12.2	890	11	10.7	146.8	35.0	0.24

* AGB data is the value measured at RB sampling year that correspond to value of YAE

** CumAGB has been or calculated or extracted from the corresponding reference

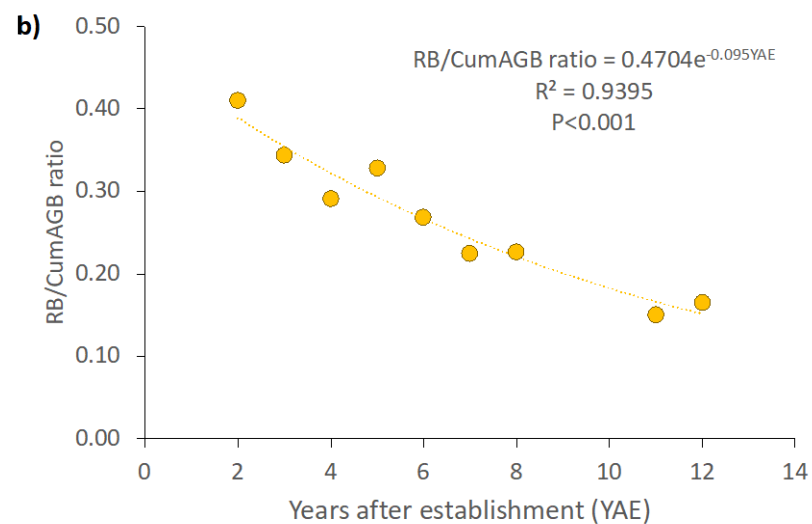
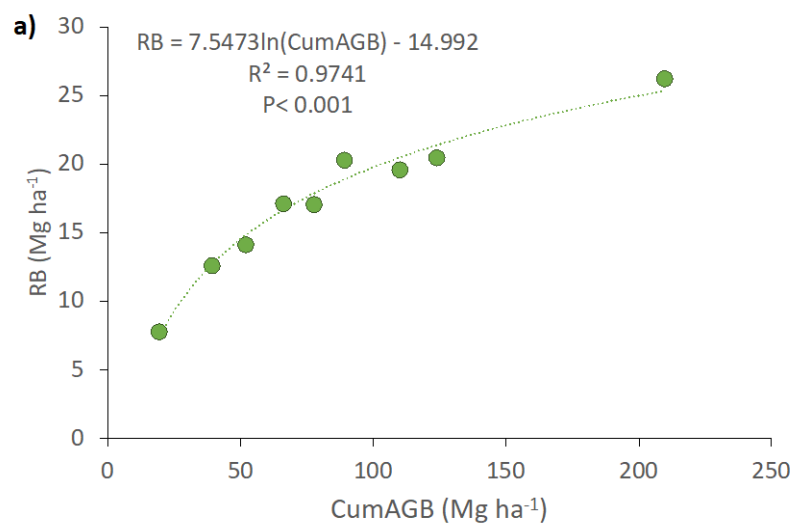
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Table S2. Average data grouped per years after establishment (YAE) of RB, CumAGB and RB/AGB ratio

YAE	n of obs	RB	Cum_AGB	RB/CumAGB ratio
years		Mg ha ⁻¹	Mg ha ⁻¹	-
2	4	7.80	19.37	0.41
3	5	12.63	39.12	0.34
4	10	14.15	51.90	0.29
5	3	17.17	66.03	0.33
6	4	17.08	77.58	0.27
7	3	19.60	110.13	0.22
8	1	20.30	89.20	0.23
11	2	26.27	209.78	0.15
12	1	20.50	124.00	0.17

Fig. S1 a) Allometric equation to estimate *Miscanthus* rhizome biomass (RB) using cumulative AGB data and **b)** the relationship between the RB/CumAGB ratio and years after establishment of *Miscanthus* (b)



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Table S3. C source and formulas to estimate the C input from novel *Miscanthus* hybrids (Mg C ha⁻¹) as adapted from Martani et al 2023

Source of C	Formulas	Hybrids	Parameters Value		
			HI	R:S	β
C from stubble (<i>C_s</i>)	$0.1 \times (Y/HI) \times 0.45$	GRC 3	0.9	0.69	0.967
C in roots (<i>Cr</i>)	$\sum_{y1-y4} [(Y/HI) \times R:S \times Turn \times 0.45 \times BG_{F-Depth}]$	GRC9	0.9	0.34	0.967
C in roots exudates (<i>C_e</i>)	$\sum_{y1-y4} [0.09 \times (Y/HI) \times 0.45 \times BG_{F-Depth}]$	GRC14	0.9	0.36	0.967
C in rhizomes (<i>Cr_{hiz}</i>)	$(Y \times 0.25 \times 0.43 \times 0.7) / 3$				
C tot	$C_s + Cr + C_e + C_w + Cr_h$				

Note:

Y: annual yield (for *Cr_{hiz}* calculation, cumulative yield was used)

HI: Harvest Index;

R:S: Root to Shoot ratio of different *Miscanthus* hybrids;

Turn: Turnover rate (0.5 for *Miscanthus* roots);

$BG_{F-Depth} = 1 - \beta^x$. where $BG_{F-Depth}$ is the amount of roots (%) from the soil surface to a certain depth (x) expressed in centimeter (es: 10 cm, 30 cm).

The β value (0.967) describes the shape of the vertical distribution of roots within the whole soil profile (Chimento and Amaducci, 2015).

For *Cr_{hiz}* calculation, 0.25 = rate RB/cumAGB; 0.43 is the C content in rhizome as C:N is lower (Beuch et al. 2000), while 0.70 is the C turnover rate. 3 are the years of plant cultivation, where the first year is not considered since in the first year of establishment RB is negligible.

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Table S4a. Analysis of variance for two soil layers of topsoil (0-10 cm and 10-30 cm): effects of site (Site), *Miscanthus* hybrids (Hybrid), and years of *Miscanthus* hybrids cultivation (Time) on measured soil physicochemical indicators.

		BD g cm ⁻³	Porosity %	SOC g kg ⁻¹	TN g kg ⁻¹	P₂O₅ g kg ⁻¹	K₂O g kg ⁻¹	P g kg ⁻¹	K g kg ⁻¹	Ca g kg ⁻¹	Mg g kg ⁻¹
Layer 0-10 cm											
Site (S)	F	117.1	124.1	122.7	29.0	122.73	43.72	97.81	36.4	62.1	1179.1
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.29	0.6	2.0	0.47	0.52	1.23	0.36	1.55	1.74	0.61
	<i>p</i>	0.83	0.61	0.12	0.70	0.67	0.30	0.78	0.20	0.16	0.61
Time	F	132.1	166.1	7.14	14.47	334.64	73.76	42.77	106.4	0.1	0.06
	<i>p</i>	<0.001	<0.001	0.09	<0.001	<0.001	<0.001	<0.001	<0.001	0.89	0.80
S x Hy	F	0.34	0.6	0.36	0.46	0.33	0.79	1.09	0.46	1.61	0.31
	<i>p</i>	0.98	0.87	0.98	0.94	0.98	0.67	0.38	0.94	0.1	0.99
S x Time	F	57.5	68.7	11.6	10.14	45.78	17.62	38.15	22.4	0.32	2.59
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.89	0.03
Hy x Time	F	0.30	0.6	2.01	0.47	0.51	0.30	0.74	0.45	0.18	0.611
	<i>p</i>	0.82	0.60	0.12	0.70	0.67	1.23	0.53	0.71	0.90	0.60
S x Hy x Time	F	0.57	0.9	0.36	0.46	0.32	0.67	1.59	0.26	0.16	0.31
	<i>p</i>	0.89	0.59	0.98	0.95	0.99	0.79	0.10	0.99	0.99	0.99
Layer 10-30 cm											
Site (S)	F	126.1	126.5	47.6	10.6	104.68	26.80	99.60	151.3	239.3	832.2
	<i>p</i>	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.03	0.03	0.51	0.21	0.24	0.26	1.13	0.44	1.28	1.43
	<i>p</i>	0.99	0.99	0.67	0.88	0.86	0.85	0.35	0.72	0.29	0.24
Time	F	9.045	9.04	4.63	2.92	331.55	36.13	15.77	332.5	1.11	10.90
	<i>p</i>	0.003	<0.001	0.034	0.09	<0.001	<0.001	<0.001	<0.001	0.29	0.014
S x Hy	F	0.6	0.60	0.21	0.44	0.30	0.54	0.95	1.07	1.12	0.67
	<i>p</i>	0.8	0.85	0.99	0.95	0.99	0.90	0.52	0.40	0.35	0.80
S x Time	F	10.6	10.4	8.10	8.65	46.07	28.10	23.32	55.5	0.14	5.76
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.97	0.002
Hy x Time	F	0.03	0.03	0.99	0.21	0.24	0.26	1.57	0.18	0.18	1.41
	<i>p</i>	0.99	0.99	0.40	0.88	0.86	0.84	0.20	0.90	0.90	0.24
S x Hy x Time	F	0.64	0.60	0.25	0.44	0.30	0.54	1.21	0.62	0.21	0.67
	<i>p</i>	0.85	0.85	0.99	0.95	0.99	0.90	0.28	0.84	0.99	0.80

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Table S4b. Analysis of variance for two soil layers of topsoil (0-10 cm and 10-30 cm): effects of site (Site), *Miscanthus* hybrids (Hybrid), and years of *Miscanthus* hybrids cultivation (Time) on measured soil biological indicators such as microbial biomass (dsDNA in ng g⁻¹ dry soil) and enzyme activities (nM 4-MUF,7-AMC g⁻¹ dry soil h⁻¹)

		C- acquiring enzymes			N- acquiring enzymes				P- acquiring enzymes					S- acq	Esterases		Redox	Microbial
		betaG	Cell	Xilo	Aprot	Argi	Chit	Leu	acP	alkP	bisP	inositP	piroP	aryS	Butir	Fda	Perox	biomass
Layer 0-10 cm																		
Site (S)	F	24.99	22.84	23.82	19.09	17.40	23.91	16.62	96.56	25.82	32.60	17.98	73.44	35.97	13.02	39.09	14.46	51.72
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.96	0.98	1.51	0.59	0.79	0.91	0.64	1.81	0.67	0.51	0.75	0.66	0.54	3.08	0.67	0.30	1.57
	p	0.42	0.41	0.22	0.62	0.50	0.44	0.59	0.16	0.57	0.67	0.53	0.58	0.65	0.03	0.57	0.83	0.21
Time	F	31.58	41.27	125.04	52.91	1.72	24.56	25.41	0.06	4.21	2.81	11.82	21.44	57.55	72.37	59.25	103.34	30.78
	p	<0.001	<0.001	<0.001	<0.001	0.19	<0.001	<0.001	0.80	0.04	0.09	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S x Hy	F	0.61	0.73	0.72	0.56	0.70	1.22	0.48	0.80	0.53	0.32	0.32	0.51	0.58	0.62	0.89	0.32	0.72
	p	0.85	0.74	0.75	0.89	0.77	0.29	0.94	0.67	0.91	0.99	0.99	0.93	0.87	0.84	0.58	0.99	0.76
S x Time	F	30.35	34.74	39.27	13.18	39.97	43.31	31.56	16.40	26.49	4.44	9.37	12.558	33.74	8.08	39.60	17.99	62.63
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hy x Time	F	0.96	0.98	1.51	0.59	0.79	0.91	0.64	1.81	0.67	0.51	0.75	0.66	0.54	3.08	0.67	0.30	1.57
	p	0.42	0.41	0.22	0.62	0.50	0.44	0.59	0.15	0.57	0.67	0.53	0.58	0.65	0.03	0.57	0.83	0.20
S x Hy x Time	F	0.61	0.73	0.72	0.56	0.70	1.22	0.48	0.80	0.53	0.32	0.32	0.51	0.58	0.62	0.89	0.32	0.72
	p	0.85	0.75	0.75	0.89	0.77	0.27	0.94	0.66	0.91	0.99	0.99	0.93	0.87	0.84	0.58	0.99	0.76
Layer 10-30 cm																		
Site (S)	F	29.48	9.61	25.79	10.98	12.60	23.24	14.31	130.62	52.22	121.67	47.68	294.038	77.95	95.54	35.21	73.65	43.55
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.95	1.13	0.48	0.02	1.17	0.09	0.71	0.96	0.34	0.64	0.56	0.30	0.77	2.07	0.02	1.53	0.50
	p	0.42	0.34	0.69	0.99	0.32	0.96	0.55	0.42	0.79	0.59	0.64	0.82	0.52	0.11	0.99	0.22	0.69
Time	F	28.40	19.95	90.57	7.79	15.63	0.01	23.19	13.38	66.65	0.76	23.17	16.76	80.05	175.19	10.60	96.52	70.33
	p	<0.001	<0.001	<0.001	0.007	<0.001	0.92	<0.001	<0.001	<0.001	0.38	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
S x Hy	F	0.65	0.58	0.69	0.73	0.72	0.55	0.69	1.27	1.01	0.65	0.59	0.82	0.89	1.41	0.49	2.01	0.39
	p	0.81	0.87	0.78	0.74	0.75	0.89	0.78	0.25	0.46	0.81	0.87	0.65	0.57	0.18	0.93	0.03	0.97
S x Time	F	12.45	5.47	9.16	2.67	9.03	4.70	12.46	2.41	14.56	26.86	24.66	58.66	14.27	9.58	1.04	6.37	10.25
	p	<0.001	<0.001	<0.001	0.03	<0.001	<0.001	<0.001	0.04	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.40	<0.001	<0.001
Hy x Time	F	0.95	1.13	0.48	0.02	1.17	0.09	0.71	0.96	0.34	0.64	0.57	0.30	0.77	2.07	0.02	1.53	0.50
	p	0.42	0.34	0.69	0.99	0.32	0.96	0.55	0.42	0.79	0.58	0.64	0.82	0.52	0.11	0.99	0.21	0.69
S x Hy x Time	F	0.65	0.58	0.69	0.73	0.72	0.55	0.69	1.26	1.01	0.66	0.59	0.82	0.89	1.41	0.46	2.01	0.39
	p	0.81	0.88	0.79	0.74	0.76	0.90	0.79	0.25	0.46	0.81	0.88	0.65	0.57	0.17	0.93	0.03	0.97

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Table S5. Correlation matrix between variations of SOC concentration (Δ SOC g kg⁻¹) and shift of EA (Δ EA nanomoles g⁻¹ dry soil h⁻¹) during four years of *Miscanthus* cultivation.

	SOC_conc	dsDNA	aryS	betaG	cell	xilo	argi	chit	aprot	leu	acP	bisP	piroP	alkP	inositP	butir	fda	perox
SOC_conc	1	0.6	0.22	0.29	0.39	0.3	0.39	0.37	0.34	0.33	0.43	-0.16	-0.12	0.28	0.01	0.26	0.41	0.46
dsDNA	0.6	1	0.42	0.57	0.57	0.58	0.63	0.45	0.31	0.59	0.5	-0.01	0.04	0.4	0.18	0.37	0.52	0.46
aryS	0.22	0.42	1	0.77	0.7	0.77	0.74	0.36	0.35	0.84	0.66	0.65	0.51	0.63	0.66	0.52	0.54	0.51
betaG	0.29	0.57	0.77	1	0.88	0.96	0.87	0.53	0.29	0.89	0.4	0.47	0.48	0.74	0.61	0.38	0.56	0.29
cell	0.39	0.57	0.7	0.88	1	0.88	0.85	0.48	0.31	0.85	0.34	0.34	0.32	0.74	0.53	0.43	0.54	0.42
xilo	0.3	0.58	0.77	0.96	0.88	1	0.86	0.45	0.34	0.88	0.4	0.46	0.49	0.76	0.6	0.43	0.5	0.37
argi	0.39	0.63	0.74	0.87	0.85	0.86	1	0.54	0.34	0.92	0.4	0.4	0.4	0.81	0.55	0.39	0.59	0.42
chit	0.37	0.45	0.36	0.53	0.48	0.45	0.54	1	0.08	0.51	0.34	0.15	0.12	0.31	0.31	-0.04	0.76	0.2
aprot	0.34	0.31	0.35	0.29	0.31	0.34	0.34	0.08	1	0.34	0.29	0.19	0.21	0.38	0.34	0.32	0.16	0.32
leu	0.33	0.59	0.84	0.89	0.85	0.88	0.92	0.51	0.34	1	0.44	0.52	0.47	0.79	0.62	0.42	0.59	0.43
acP	0.43	0.5	0.66	0.4	0.34	0.4	0.4	0.34	0.29	0.44	1	0.22	0.12	0.16	0.21	0.51	0.58	0.66
bisP	-0.16	-0.01	0.65	0.47	0.34	0.46	0.4	0.15	0.19	0.52	0.22	1	0.79	0.58	0.78	0.21	0.16	0.11
piroP	-0.12	0.04	0.51	0.48	0.32	0.49	0.4	0.12	0.21	0.47	0.12	0.79	1	0.56	0.75	0.1	0.03	-0.01
alkP	0.28	0.4	0.63	0.74	0.74	0.76	0.81	0.31	0.38	0.79	0.16	0.58	0.56	1	0.71	0.31	0.3	0.31
inositP	0.01	0.18	0.66	0.61	0.53	0.6	0.55	0.31	0.34	0.62	0.21	0.78	0.75	0.71	1	0.19	0.25	0.15
butir	0.26	0.37	0.52	0.38	0.43	0.43	0.39	-0.04	0.32	0.42	0.51	0.21	0.1	0.31	0.19	1	0.22	0.56
fda	0.41	0.52	0.54	0.56	0.54	0.5	0.59	0.76	0.16	0.59	0.58	0.16	0.03	0.3	0.25	0.22	1	0.38
perox	0.46	0.46	0.51	0.29	0.42	0.37	0.42	0.2	0.32	0.43	0.66	0.11	-0.01	0.31	0.15	0.56	0.38	1

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Table S6. Variations of EA from T₀ to T₄ sampling time expressed as Δ EA nanomoles g⁻¹ dry soil h⁻¹

Site	Depth	dsDNA	aryS	betaG	cell	xilo	argi	chit	aprot	leu	acP	bisP	piroP	alkP	inositP	butir	fda	perox
CHV	0-10 cm	15.99	2.20	0.01	0.29	0.45	4.82	0.08	-2.59	8.42	2.93	4.02	1.47	24.39	0.11	133.80	-0.24	2.92
	10-30 cm	9.61	0.51	0.75	0.07	0.20	1.85	0.15	-11.67	2.91	0.51	0.05	0.00	10.97	0.00	54.37	-0.11	0.95
OLI	0-10 cm	-6.87	-1.91	0.21	0.11	0.26	0.38	-1.22	-7.90	-3.14	-12.46	-1.41	1.71	23.47	0.09	22.76	-1.45	0.54
	10-30 cm	-1.75	4.04	1.69	0.12	0.51	2.76	-0.32	-1.94	6.36	1.21	7.82	8.85	35.64	0.94	61.67	-0.65	0.93
PAC 1	0-10 cm	17.76	3.47	6.77	0.41	1.24	6.69	1.34	-19.37	13.90	4.64	2.83	5.32	18.38	0.21	139.20	0.17	0.89
	10-30 cm	14.93	2.52	4.11	0.13	0.69	3.78	0.71	0.24	10.01	2.91	1.42	4.00	8.25	0.12	74.24	0.02	0.88
PAC 2	0-10 cm	-29.13	-5.37	-11.76	-1.26	-1.83	-20.57	-3.98	-35.44	-24.55	-4.99	-2.42	-3.01	-57.90	-0.62	-12.53	-1.43	-0.47
	10-30 cm	2.70	0.61	0.82	-0.02	0.16	-1.27	-0.02	-9.71	-0.07	0.45	0.33	0.32	-0.23	0.04	28.97	-0.03	1.07
TWS	0-10 cm	6.28	3.09	0.91	0.12	0.58	-2.22	-6.46	1.13	3.70	-0.21	3.86	4.03	19.20	0.21	239.92	-2.05	1.97
	10-30 cm	26.63	-1.73	-1.97	-0.10	-0.01	-1.31	-0.95	4.59	-4.40	8.10	-5.37	-1.92	3.91	-0.28	158.29	-0.42	3.16
ZAG	0-10 cm	18.80	2.83	3.73	0.33	0.83	5.69	0.30	-3.48	9.60	8.94	-1.17	-0.06	3.50	0.11	158.07	0.47	2.23
	10-30 cm	11.56	1.33	1.27	0.10	0.27	0.37	-0.39	-8.25	1.94	3.53	-2.44	-4.95	7.81	0.11	119.13	-0.08	1.45

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Table S7a. Analysis of variance for two soil layers of topsoil (0-10 cm and 10-30 cm): effects of site (Site), *Miscanthus* hybrids (Hybrid), and years of *Miscanthus* hybrids cultivation (Time) on calculated ESM of SOC and soil nutrients (Mg ha⁻¹).

		SOC	TN	P₂O₅	K₂O	P	K	Ca	Mg
		Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹
Layer 0-10 cm									
Site (S)	F	43.4	345.3	155.96	59.74	19.96	95.4	449.5	1882.3
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	1.83	0.24	0.56	0.82	0.49	1.0	1.24	0.97
	<i>p</i>	0.14	0.86	0.65	0.49	0.69	0.39	0.30	0.41
Time	F	4.34	19.3	319.09	94.5	33.93	52.4	44.56	0.53
	<i>p</i>	0.04	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.46
S x Hy	F	0.28	0.54	0.35	0.65	0.779	0.41	1.27	0.70
	<i>p</i>	0.99	0.90	0.99	0.82	0.70	0.96	0.25	0.77
S x Time	F	12.34	13.67	44.10	18.81	33.19	17.26	15.7	1.38
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.26
Hy x Time	F	1.77	0.19	0.55	0.77	0.51	0.66	0.10	1.01
	<i>p</i>	0.15	0.90	0.65	0.51	0.68	0.57	0.95	0.39
S x Hy x Time	F	0.29	0.56	0.34	0.63	0.75	0.48	0.43	0.70
	<i>p</i>	0.99	0.89	0.99	0.84	0.72	0.94	0.96	0.77
Layer 10-30 cm									
Site (S)	F	25.6	12.04	144.94	47.63	72.82	171.1	674.4	1409.3
	<i>p</i>	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.34	0.12	0.25	0.63	1.14	0.51	0.94	1.35
	<i>p</i>	0.79	0.94	0.85	0.59	0.34	0.67	0.42	0.27
Time	F	10.69	26.73	330.0995	17.44	19.72	306.6	35.94	9.83
	<i>p</i>	0.0017	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S x Hy	F	0.20	0.41	0.30	0.41	1.16	0.80	0.61	0.59
	<i>p</i>	0.99	0.96	0.99	0.96	0.36	0.66	0.84	0.86
S x Time	F	6.19	11.13	46.50	23.66	36.14	36.95	6.55	6.43
	<i>p</i>	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Hy x Time	F	0.94	0.15	0.26	0.56	1.70	0.12	0.30	1.31
	<i>p</i>	0.42	0.92	0.85	0.63	0.18	0.94	0.82	0.27
S x Hy x Time	F	0.23	0.40	0.30	0.39	1.55	0.32	0.22	0.60
	<i>p</i>	0.99	0.97	0.99	0.97	0.11	0.99	0.99	0.85

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Table S7b. Analysis of variance for the topsoil layer (0-30 cm): effects of site (Site), *Miscanthus* hybrids (Hybrid), and years of *Miscanthus* hybrids cultivation (Time) on calculated ESM of SOC and soil nutrients (Mg ha⁻¹).

		SOC	TN	P₂O₅	K₂O	P	K	Ca	Mg
		Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹
Layer 0-30 cm									
Site (S)	F	46.07	17.23	149.19	49.94	79.53	322.3	682.3	1861.2
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (Hy)	F	0.97	0.01	0.29	0.34	0.29	0.61	1.02	0.83
	<i>p</i>	0.41	0.99	0.82	0.79	0.83	0.60	0.36	0.48
Time	F	0.34	4.42	348.94	0.94	43.39	439.3	47.52	11.2
	<i>p</i>	0.56	0.03	<0.001	0.33	<0.001	<0.001	<0.001	0.0013
S x Hy	F	0.25	0.53	0.30	0.45	0.58	0.53	0.80	0.39
	<i>p</i>	0.99	0.90	0.99	0.95	0.87	0.91	0.67	0.97
S x Time	F	7.99	7.24	47.51	22.63	29.67	53.6	10.2	6.25
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Hy x Time	F	1.47	0.01	0.29	0.26	0.30	0.40	0.25	0.85
	<i>p</i>	0.23	0.99	0.82	0.85	0.82	0.74	0.85	0.47
S x Hy x Time	F	0.34	0.53	0.30	0.42	0.58	0.47	0.22	0.39
	<i>p</i>	0.98	0.91	0.99	0.96	0.88	0.94	0.99	0.97

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Table S8. Analysis of variance for the topsoil layer (0-30 cm): effects of site (Site) and *Miscanthus* hybrids (Hybrid) on rate of SOC and soil nutrients stock variation ($\text{Mg ha}^{-1} \text{y}^{-1}$).

		SOC	TN	P₂O₅	K₂O	P	K	Ca	Mg
		$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$	$\text{Mg ha}^{-1} \text{y}^{-1}$
Layer 0-30 cm									
Site (S)	F	4.89	0.87	15.65	11.06	11.07	18.82	0.03	1.75
	<i>p</i>	0.0074	0.52	<0.001	<0.001	<0.001	<0.001	0.99	0.18
Hybrid (Hy)	F	2.01	0.03	0.31	0.47	0.23	0.50	0.35	1.02
	<i>p</i>	0.12	0.99	0.81	0.70	0.87	0.69	0.78	0.39
Site x Hybrid	F	0.46	1.58	1.11	1.17	0.99	1.19	0.37	0.52
	<i>p</i>	0.94	0.11	0.36	0.32	0.47	0.30	0.98	0.91

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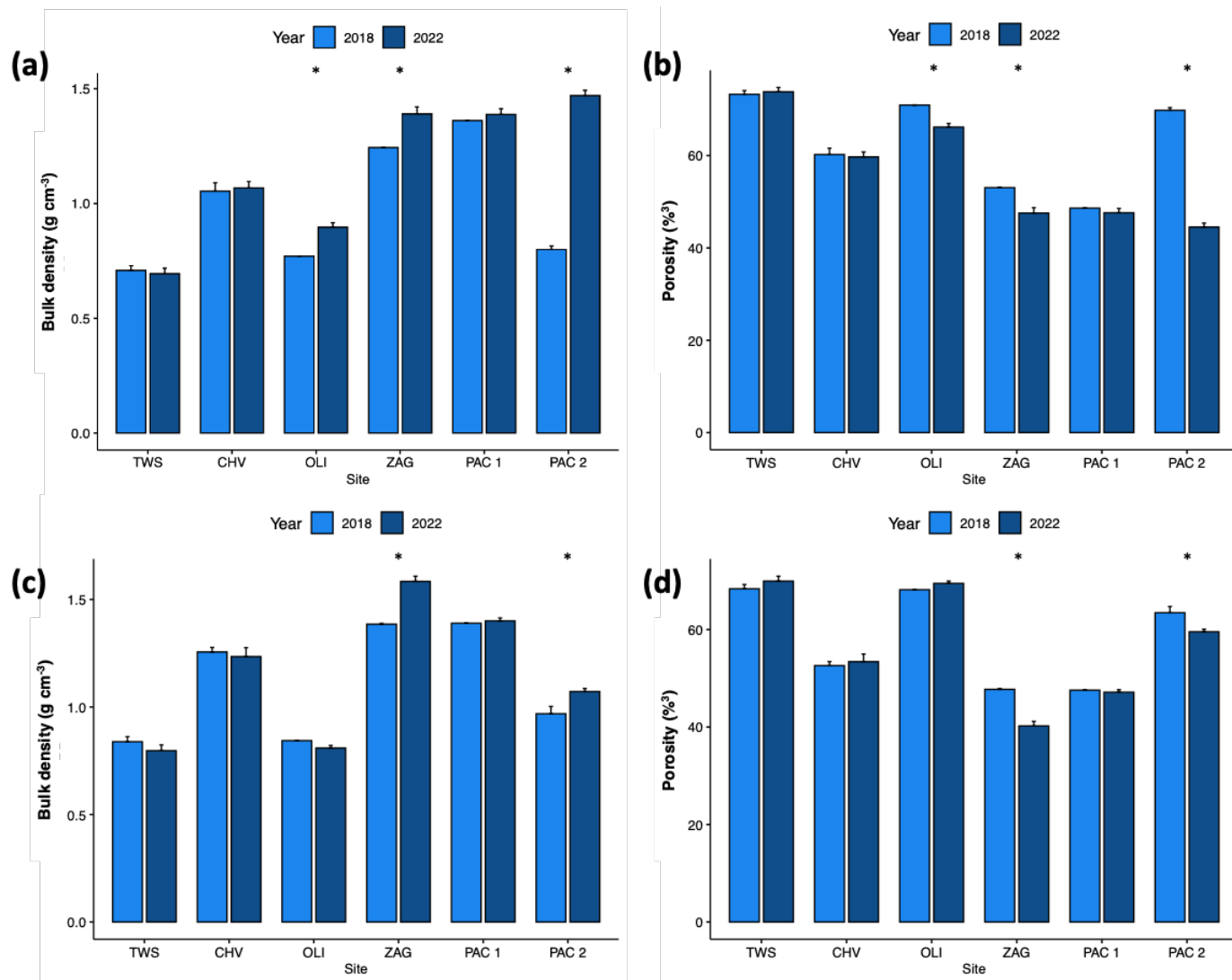
Table S9. Contribution (%) to the total C input to soil (Mg ha^{-1}) of the following *Miscanthus* organs: C from stubble (*Cs*), C from roots (*Cr*), C from root exudates (*Ce*), and C from rhizomes (*Crhiz*).

Site	Hybrid	<i>Cs</i>	<i>Cr</i>	<i>Ce</i>	<i>Crh</i>
TWS	GRC 14	19.30	41.30	20.74	18.56
	GRC 15	11.97	47.05	21.60	19.32
	GRC 3	10.49	59.99	15.55	13.91
	GRC 9	22.74	38.33	20.49	18.33
CHV	GRC 14	12.90	44.60	22.40	20.04
	GRC 15	12.48	46.78	21.47	19.21
	GRC 3	10.51	59.98	15.55	13.91
	GRC 9	19.47	0.71	42.08	37.65
OLI	GRC 14	7.81	47.22	23.72	21.22
	GRC 15	12.03	46.11	21.16	20.64
	GRC 3	9.57	59.82	15.51	15.06
	GRC 9	12.45	41.62	22.25	23.62
ZAG	GRC 14	15.65	43.18	21.69	19.40
	GRC 15	12.25	46.90	21.53	19.26
	GRC 3	10.68	59.86	15.52	13.88
	GRC 9	16.64	41.37	22.12	19.79
PAC 1	GRC 14	9.43	46.39	23.30	20.84
	GRC 15	15.01	39.72	23.85	21.34
	GRC 3	8.92	56.27	18.35	16.42
	GRC 9	11.81	47.63	21.38	19.13
PAC 2	GRC 14	9.38	57.39	17.51	15.67
	GRC 15	11.34	50.61	20.05	17.94
	GRC 3	10.09	53.57	19.15	17.14
	GRC 9	13.80	42.79	22.88	20.47
		12.11	46.63	21.57	19.63

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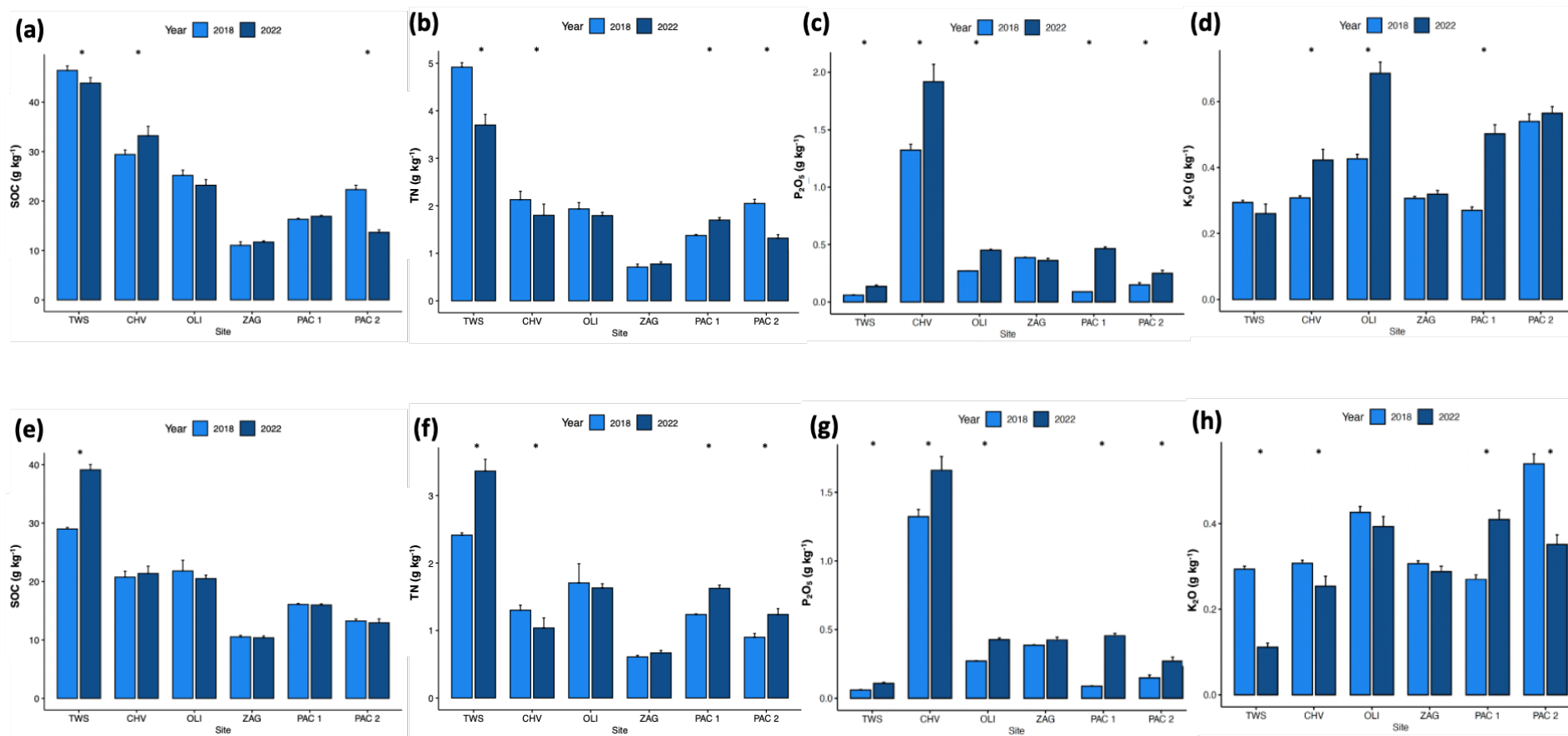
Figure S2. Mean values (n=4) of bulk density in 0-10 (a) and 10-30 (c) cm soil layers, and soil porosity in 0-10 (b) and 10-30 (d) cm soil layers among 6 European marginal sites at T₀ (light blue) and T₄ (dark blue). (*) show statistically different variations from T₀ to T₄ (Tukey's test, P: 0.05) within the same site.



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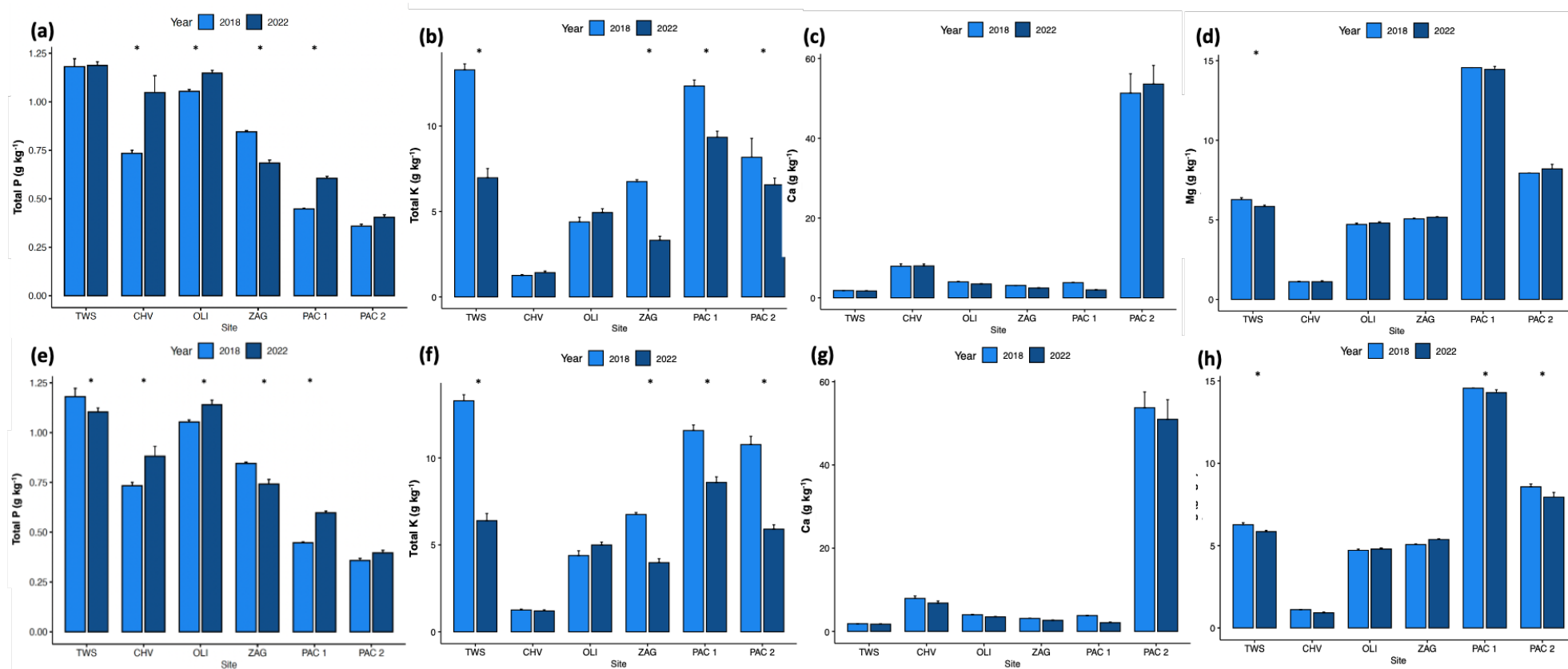
Figure S3. Mean values (n=4) of SOC, TN, P₂O₅ and K₂O concentration (g kg⁻¹) in 0-10 cm (a, b, c, d) and 10-30 cm (e, f, g, h) soil layers among 6 European marginal sites at T₀ (light blue) and T₄ (dark blue). (*) show statistically different variations from T₀ to T₄ (Tukey's test, P: 0.05) within the same site.



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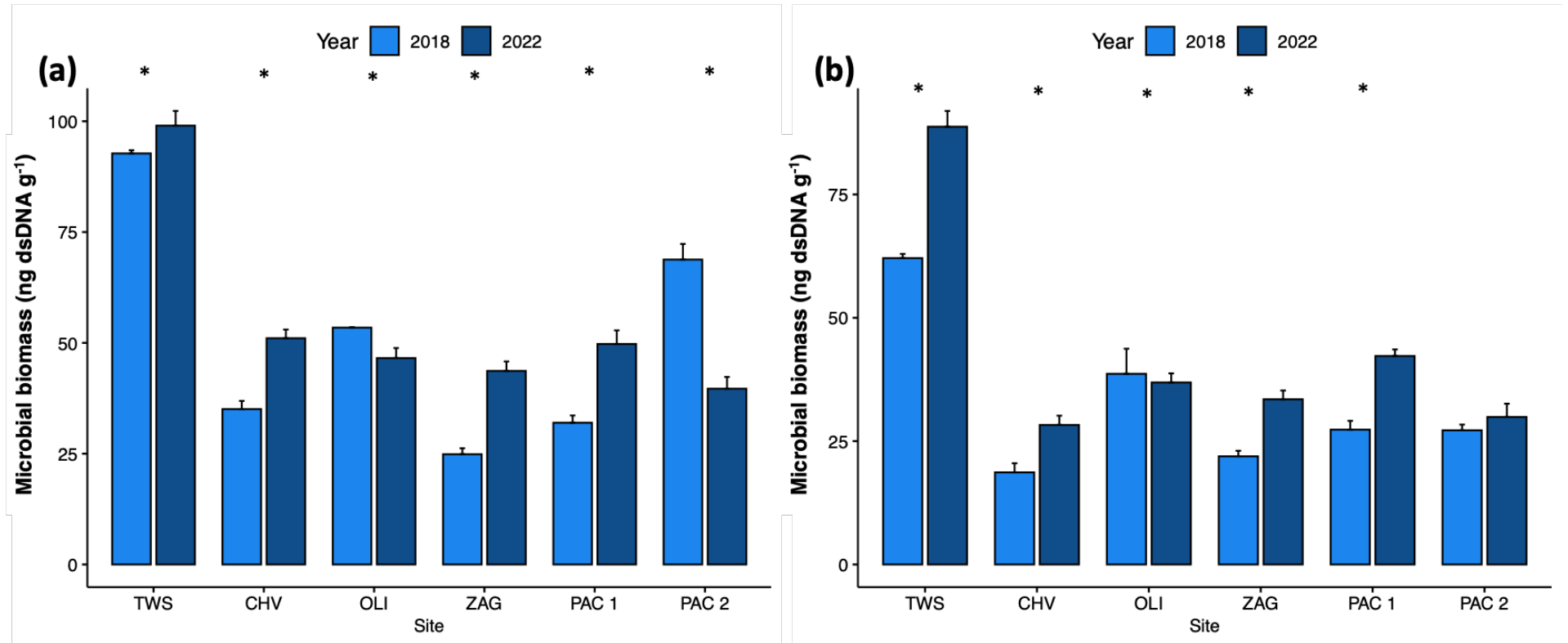
Figure S4. Mean values (n=4) of TP, TK, Ca and Mg concentration (g kg^{-1}) in 0-10 cm (a, b, c, d) and 10-30 cm (e, f, g, h) soil layers among 6 European marginal sites at T₀ (light blue) and T₄ (dark blue). (*) show statistically different variations from T₀ to T₄ (Tukey's test, P: 0.05) within the same site.



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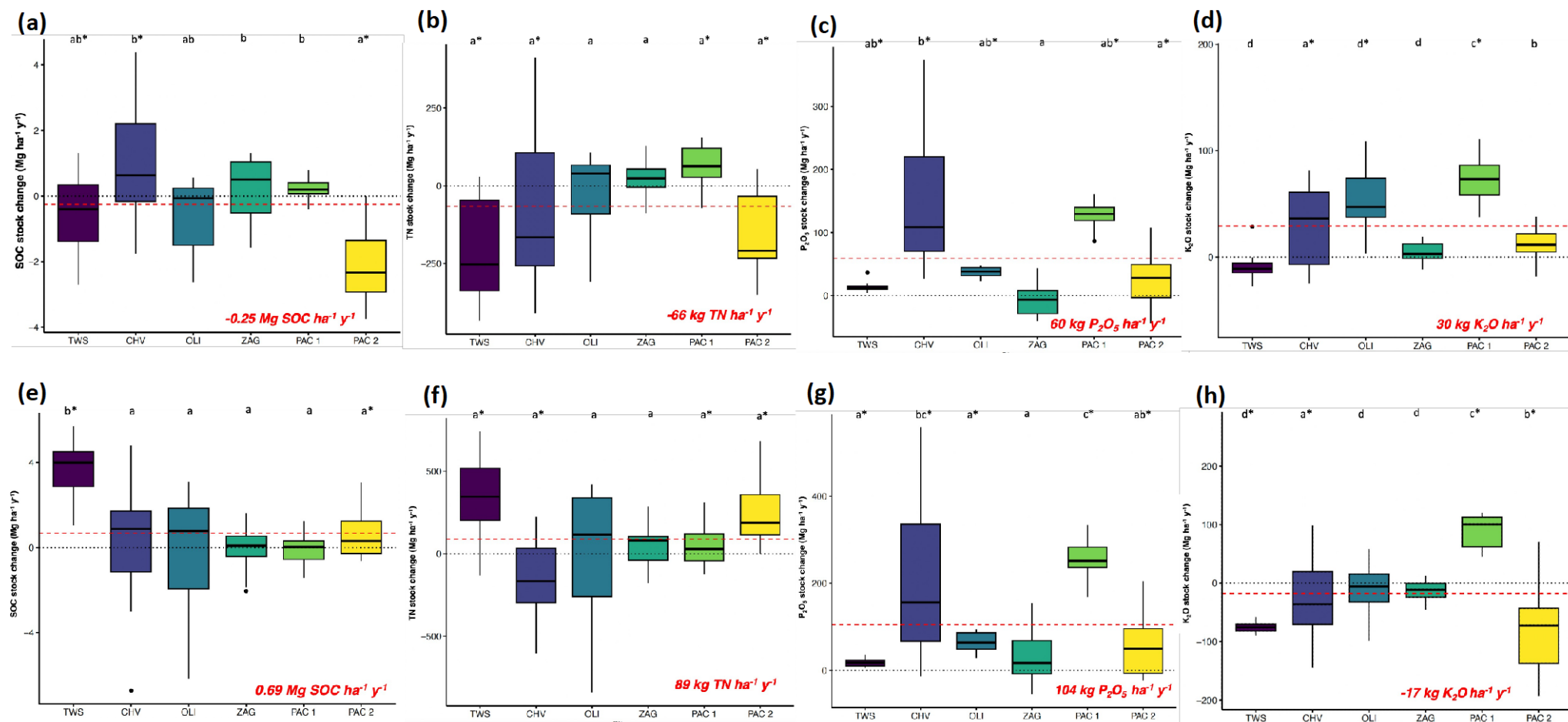
Figure S5. Mean values (n=4) of microbial biomass measured as dsDNA (ng g⁻¹_{dry soil}) in 0-10 cm (a) and 10-30 cm (b) soil layers among 6 European marginal sites at T₀ (light blue) and T₄ (dark blue). (*) show statistically different variations from T₀ to T₄ (Tukey's test, P: 0.05) within the same site.



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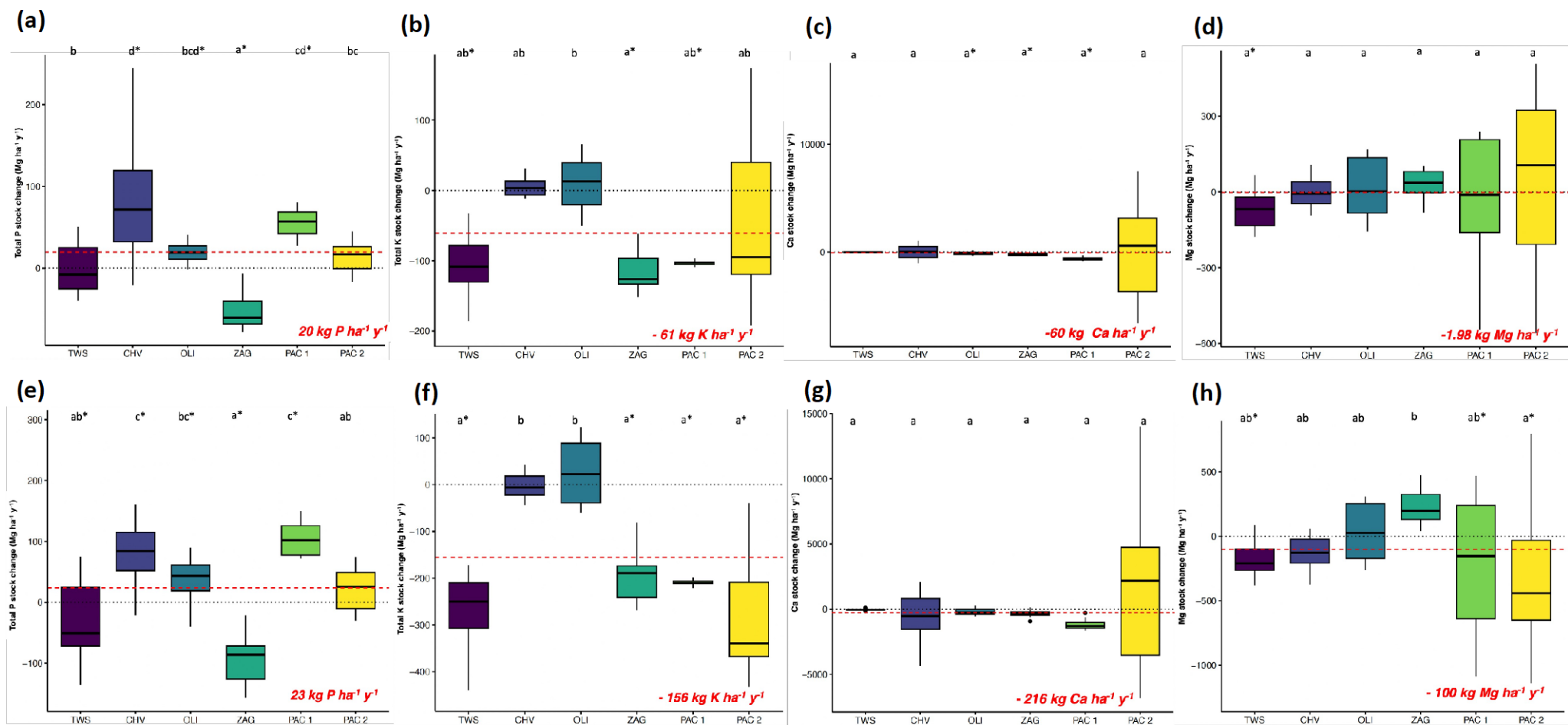
Figure S6. Rate of SOC and soil nutrients stock variation (Δ stock $\text{Mg ha}^{-1} \text{y}^{-1}$) in the 0-10 cm (a, b, c, d) and 10-30 cm (e, f, g, h) soil layers, in 4 years of *Miscanthus* cultivation. Different letters denote statistically different means among marginal sites (Tukey's test, $P < 0.05$), while (*) show statistically different variations from T_0 to T_4 (Tukey's test, $P < 0.05$) within the same site.



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Figure S7. Rate of SOC and soil nutrients stock variation (Δ stock $\text{Mg ha}^{-1} \text{y}^{-1}$) in the 0-10 cm (a, b, c, d) and 10-30 cm (e, f, g, h) soil layers, in 4 years of *Miscanthus* cultivation. Different letters denote statistically different means among marginal sites (Tukey's test, $P: 0.05$), while (*) show statistically different variations from T_0 to T_4 (Tukey's test, $P: 0.05$) within the same site.



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