

Supplementary information

Lower mineralizability of soil carbon with higher legacy soil moisture

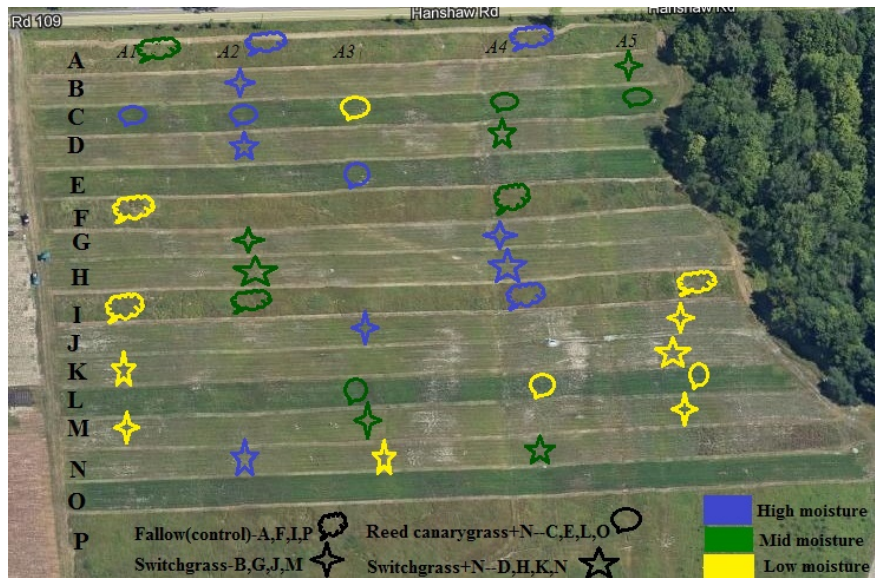
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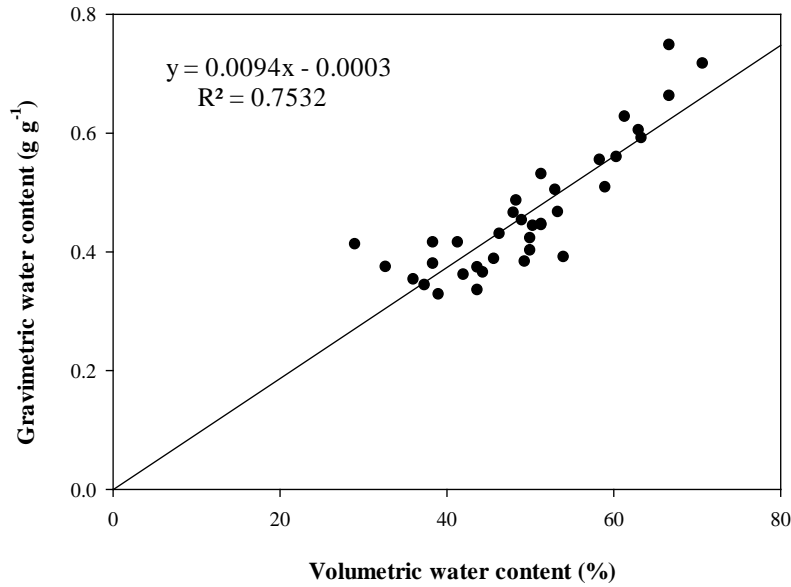
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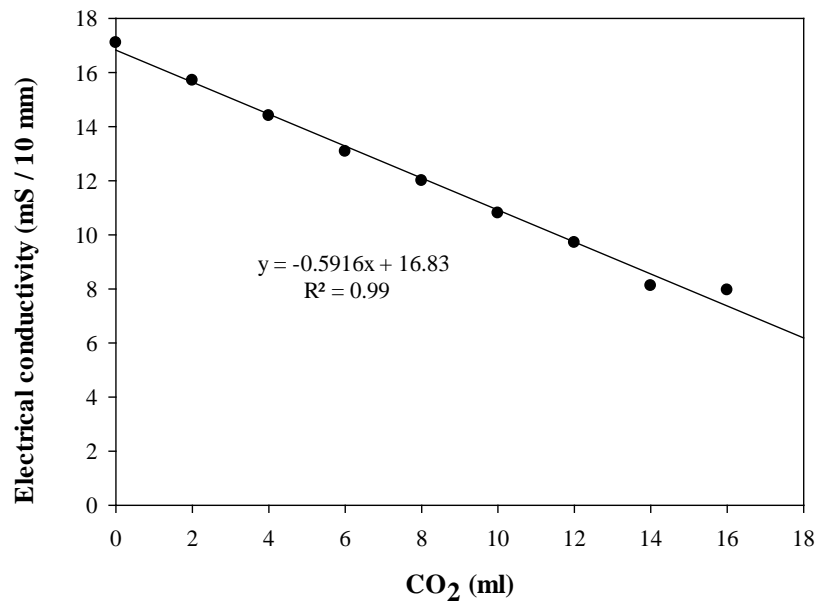
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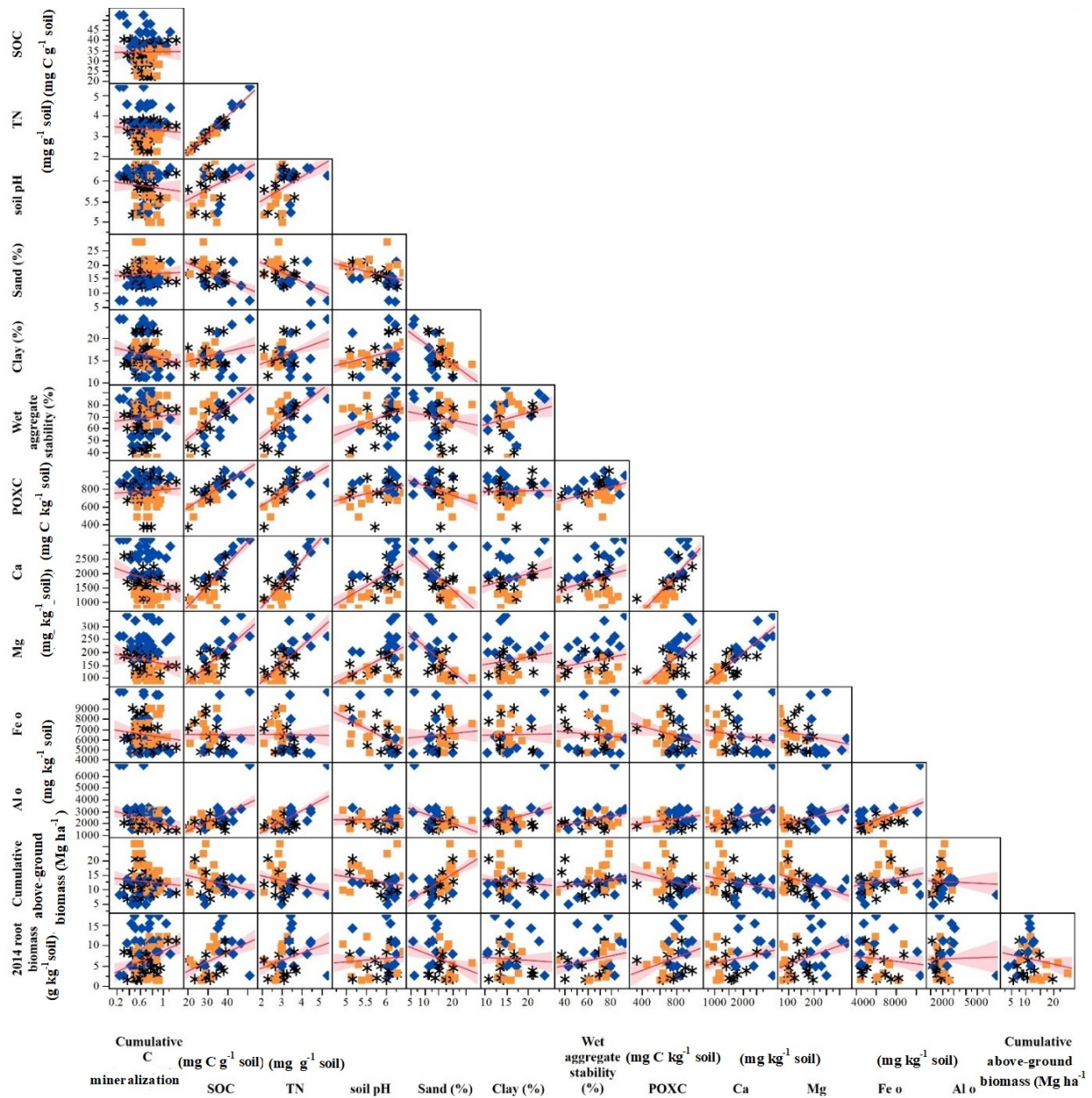
Supplementary Figure S1. Google Earth depicting experimental plots varying in moisture levels (colors) and cropping systems (symbols). Subplot ID's are shown in Table S1.



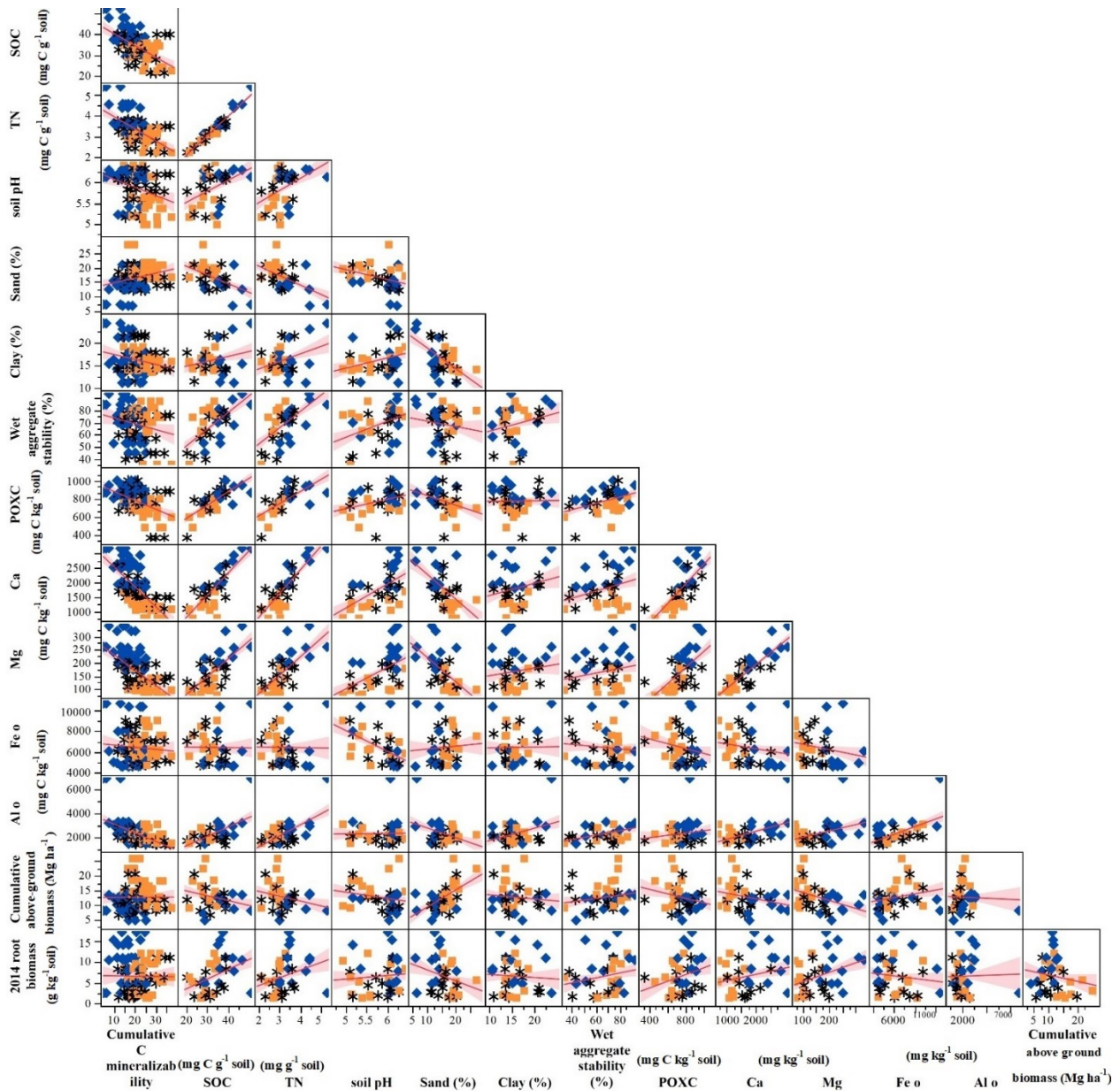
Supplementary Figure S2. Gravimetric water content as a function of volumetric water content for soils from the subset of chosen subplots (n=36) for the sampling day in Aug 2014. Linear equation used for estimating long-term equivalent gravimetric content of three wetness levels and subsequent laboratory water adjustments.



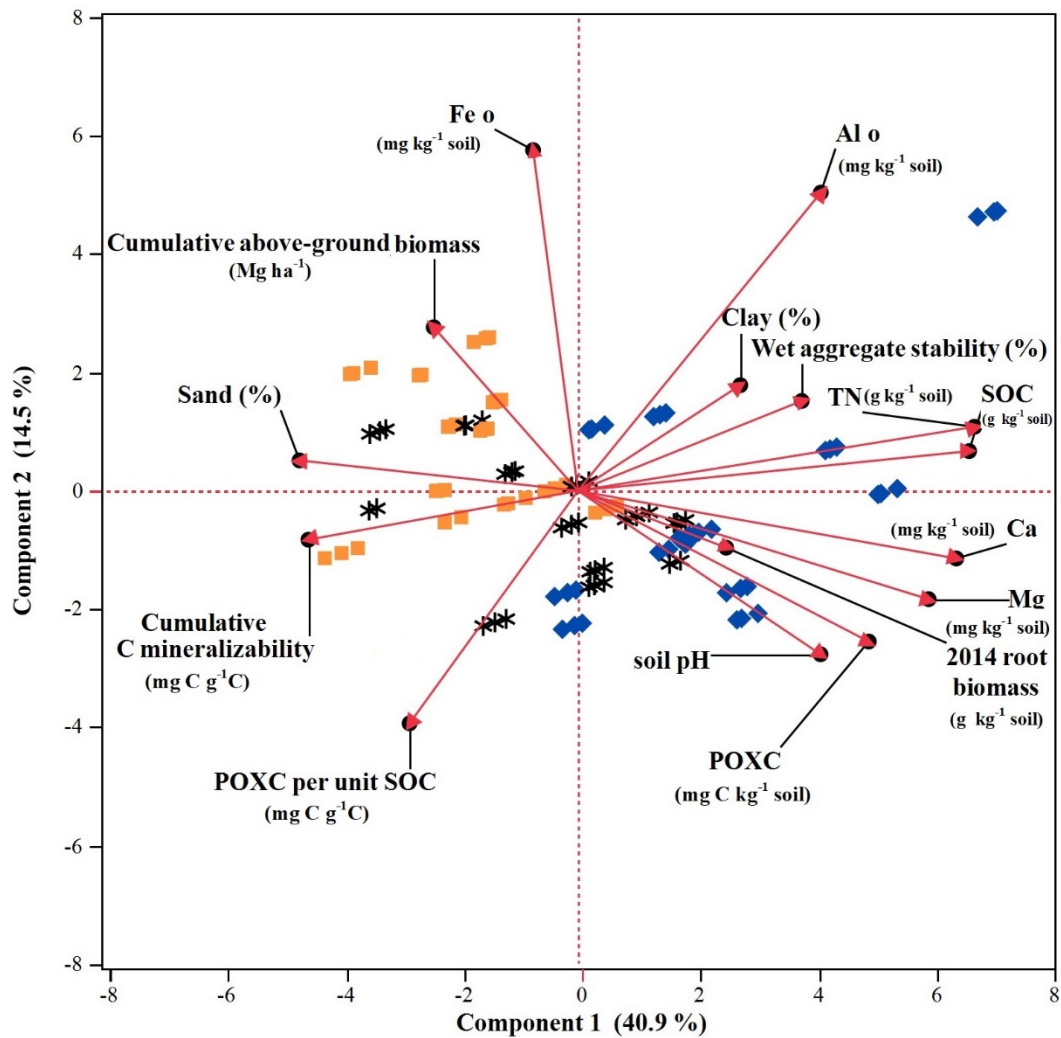
Supplementary Figure S3. Standard graph regression for determination of electrical conductivity value at known CO₂ volume for use in calibration.



Supplementary Figure S4a. Scatterplot matrix for cumulative C mineralization (mg C g⁻¹ soil) at 42 days to soil properties and cumulative crop growth parameters. Blue diamonds, black stars and orange squares represent samples from high, mid, and low field moisture levels, respectively.



Supplementary Figure S4b. Scatterplot matrix for cumulative C mineralizability (mg C g⁻¹ C) at 42 days to soil properties and cumulative crop growth parameters. Blue diamonds, black stars and orange squares represent root samples from high, mid, and low field moisture levels, respectively.



Supplementary Fig. S5. Biplot of the first two principal components of variability among soil properties, crop-growth properties and C mineralizability. Red vectors represent principal component loadings of each variable. Blue diamonds, black stars and orange squares represent samples from high, mid, and low field moisture levels, respectively.

Supplementary Table S1. Subplot IDs of different moisture content and distribution of the four cropping systems. Equivalent water content values of long term high, mid and low volumetric wet quintile ranks.

Cropping system	Field-moisture level		
	High	Mid	Low
	Subplot IDs		
Fallow-Control	A2	A1	F1
	A4	I2	I1
	I4	F4	I5
RCG	C2	C4	C3
	E3	L3	L5
	C1	C5	L4
SWG	B2	B5	J5
	G4	M3	M5
	J3	G2	M1
SWG N	D2	D4	K1
	H4	N4	K5
	N2	H2	N3
Moisture values			
	Long term volumetric high value (%)	Long term volumetric mid value (%)	Long term volumetric low value (%)
	53.94	43.1	34.9
	Long term equivalent high-water content g/g	Long term equivalent mid -water content g/g	Long term equivalent low-water content g/g
	0.5	0.4	0.3

Supplementary Table S2a. Fixed effects and their interaction for the response variable cumulative CO₂ evolution per unit soil (mg C g⁻¹ soil) at 42 days.

Effect tests			
	DF	F Ratio	P value
Field moisture level	2	0.4	0.7
Laboratory moisture level	2	91.4	<0.0001
Cropping system	3	5.3	0.005
Field moisture level *Laboratory adjustment	4	5.3	0.001

Supplementary Table S2b. Post-hoc comparisons of least square means for main effect lab moisture level and cropping system. Post-hoc comparisons of least square means for the interaction Field moisture* laboratory adjustment not shown.

Level	Least Square Mean
lab-high A	0.9
lab-mid B	0.7
lab-low C	0.6

Level	Least Square Mean
Fallow A	0.8
Switchgrass+N A B	0.7
Reed canarygrass+N A B	0.7
Switchgrass B	0.6

Supplementary Table S3a. Fixed effects and their interaction for the response variable cumulative CO₂ evolution per unit SOC (mg C g⁻¹ C) at 42 days .

Effect tests			
	DF	F Ratio	P value
Field moisture level	2	7.7	0.002
Laboratory moisture level	2	96.1	<0.0001
Cropping system	3	0.5	0.7
Field moisture level *Laboratory moisture adjustment	6	2	0.1

Supplementary Table S3b. Post-hoc comparisons of least square means of cumulative CO₂ evolution per unit SOC (mg C g⁻¹ C) for main effect field moisture and lab moisture at 42 days.

Level		Least Square Mean
lab-high	A	25.2
lab-mid	A B	21.8
lab-low	B	18.6

Level		Least Square Mean
Low	A	25.5
Mid	B	22.5
High	C	17.7

Supplementary Table S4a. Fixed effects and their interaction for the response variable slow mineralizable pool C_2 (mg C g⁻¹ SOC) at 42days, considering constant $k_1 = 0.3 \text{ day}^{-1}$.

Effect tests			
	DF	F Ratio	P value
Field moisture level	2	44	<0.0001
Laboratory moisture level	2	2	0.2
Cropping system	3	18.4	<0.0001
Field moisture level *Cropping system	6	8.6	0.0002
Field moisture level *Lab moisture level	4	1.1	0.4

Supplementary Table S4b. Post-hoc comparisons of least square means for main effect field moisture and lab moisture level.

Level (Field moisture level)			Least Square Mean
High	A		997.1
Mid		B	996.4
Low		C	995.3

Level (Cropping system)			Least Square Mean
Fallow	A		997
RCG	A		996.8
SWG		B	996.1
SWGN		B	995.4

Supplementary Table S4c. Post-hoc comparisons of least square means for the interaction Field moisture* cropping system at 42 days for C_2 , $\alpha = 0.05$ Tukey's HSD used to correct for multiple comparisons.

Level (Field moisture level *Cropping system)			Least Square Mean
Mid, RCG	A		997.6
High, RCG	A		997.3
High, SWGN	A		997.3
Mid, Fallow	A		997.1
High, Fallow	A		997.1
High, SWG	A	B	997
Mid, SWG	A	B	996.6
Low, Control	A	B	996.6
Low, RCG		B C	995.5
Low, SWG		C	994.7
Mid, SWGN		C	994.5
Low, SWGN		C	994.5

Supplementary Table S5a. Fixed effects and their interaction for the response variable MRT₂ in years (considering constant $k_1 = 0.3 \text{ day}^{-1}$)

Effect tests			
	DF	F Ratio	P value
Field moisture level	2	76.2	<0.0001
Laboratory moisture level	2	75.1	<0.0001
Cropping system	3	8.7	0.0009
Field moisture level *Laboratory moisture adjustment	4	10.5	0.0001
Field moisture level *Cropping system	6	3.9	0.01

Supplementary Table S5b. Post-hoc comparisons of least square means for main effects field moisture, lab moisture and cropping system.

Level (Field moisture level)		Least Square Mean
High	A	8
Mid	B	6.2
Low	B	5.5
Level (Lab moisture)		Least Square Mean
low	A	7.9
mid	B	6.4
mid	C	5.4
Level (Cropping system)		Least Square Mean
SWG	A	7.2
RCG	A B	6.5
SWG _N	A B	6.5
Control	B	6

Supplementary Table S5c. Post-hoc comparisons of least square means for the interaction Field moisture level* lab moisture adjustment at 42 days for C₂, $\alpha = 0.05$ Tukey's HSD used to correct for multiple comparisons.

Level (Field moisture level *Laboratory adjustment)		Least Square Mean
High, lab-low	A	10.4
High, lab-mid	B	7.5
Mid, lab-low	B C	7.4
High, lab-high	C D	6.2
Mid, lab-mid	D E	6.1
Low, lab-low	D E	6.1
Low, lab-mid	D E	5.7
Mid, lab-high	D E	5.1
Low, lab-high	E	4.8

Supplementary Table S5d. Post-hoc comparisons of least square means for the interaction Field moisture level* cropping system at 42 days for C₂, $\alpha = 0.05$ Tukey's HSD used to correct for multiple comparisons.

Level (Field moisture level *Cropping system)		Least Square Mean
High, SWG	A	8.8
High, SWGN	A B	8.1
High, Fallow	A B	7.6
High, RCG	A B	7.6
Mid, SWG	B C	7.1
Mid, RCG	B C	7
Low, SWGN	C D	5.8
Low, SWG	C D	5.8
Mid, SWGN	C D	5.6
Low, Fallow	D	5.4
Mid, Fallow	D	5.1
Low, RCG	D	5.1

Supplementary Table S6. Carbon mineralization kinetics of soil after incubation for 42 days at 25°C for the different cropping systems when rates of the rapidly mineralizing pool (k_1) were fixed to an average value (Mean+SE, $n = 3$ replicates each using technical duplicates, for the three laboratory level adjustments of three field moisture levels of each cropping system). Pool sizes and decay rates of cumulative soil C mineralization per unit SOC using double exponential model $C_{\text{cumulative}} = C_1 (1 - \exp(-k_1 x)) + (1000 - C_1) (1 - \exp(-k_2 x))$, where C_1 is the fast pool, C_2 is the slow pool and k_1 and k_2 are the first-order decomposition rate coefficients for fast and slow pool respectively, the parameter constraints chosen, $k_1 > 0$, $k_2 > 0$ and $C_1 + C_2 = 1000$. Curve fitting was performed using a k_1 value fixed to an average of all cropping systems and moisture classes of 0.3 day^{-1} . MRT_1 is the mean residence time of the fast-mineralizing pool in days ($\text{MRT}_1 = 1/k_1$) while MRT_2 is the mean residence time of slow-mineralizing pool in years ($\text{MRT}_2 = (1/k_2)/365$).

Cropping system	Field	Lab	C_1 (mg C g ⁻¹ C)	k_1	C_2 (mg C g ⁻¹ C)	k_2 (day ⁻¹)	MRT_1	MRT_2
Fallow	high	high	2.9±0.1	0.3	997.1±0.1	0.00045±0.000	3.3	6.1
	high	mid	3.4±0.4	0.3	996.6±0.4	0.00042±0.000	3.3	6.6
	high	low	2.5±0.1	0.3	997.5±0.1	0.00027±0.000	3.3	10.0
	mid	high	2.7±0.4	0.3	997.3±0.4	0.00064±0.000	3.3	4.3
	mid	mid	3.1±0.3	0.3	996.9±0.3	0.00054±0.000	3.3	5.1
	mid	low	2.9±0.1	0.3	997.1±0.1	0.00046±0.000	3.3	6.0
	low	high	3.5±0.2	0.3	996.5±0.2	0.00062±0.000	3.3	4.4
	low	mid	3.4±0.2	0.3	996.6±0.2	0.00055±0.000	3.3	5.0
Reed canarygrass + N	low	low	3.4±0.1	0.3	996.6±0.1	0.00041±0.000	3.3	6.7
	high	high	3.2±0.3	0.3	996.8±0.3	0.00046±0.000	3.3	6.0
	high	mid	2.5±0.1	0.3	997.5±0.1	0.00040±0.000	3.3	6.8
	high	low	2.4±0.1	0.3	997.6±0.1	0.00028±0.000	3.3	9.9
	mid	high	2.8±0.1	0.3	997.2±0.1	0.00046±0.000	3.3	6.0
	mid	mid	2.4±0.1	0.3	997.6±0.1	0.00043±0.000	3.3	6.4
	mid	low	2.1±0.1	0.3	997.9±0.1	0.00032±0.000	3.3	8.5
	low	high	5.0±0.3	0.3	995.0±0.3	0.00064±0.000	3.3	4.3
Switchgrass	low	mid	4.1±0.3	0.3	995.9±0.3	0.00054±0.000	3.3	5.1
	low	low	4.4±0.3	0.3	995.6±0.3	0.00046±0.000	3.3	5.9
	high	high	3.7±0.3	0.3	996.3±0.3	0.00043±0.000	3.3	6.4
	high	mid	3.0±0.2	0.3	997.0±0.2	0.00031±0.000	3.3	8.9
	high	low	2.6±0.1	0.3	997.4±0.1	0.00024±0.000	3.3	11.2
	mid	high	2.9±0.1	0.3	997.1±0.1	0.00047±0.000	3.3	5.8
	mid	mid	3.4±0.1	0.3	996.6±0.1	0.00040±0.000	3.3	6.9
	mid	low	3.9±0.2	0.3	996.1±0.2	0.00032±0.000	3.3	8.6
Switchgrass + N	low	high	5.8±0.2	0.3	994.2±0.2	0.00055±0.000	3.3	5.0
	low	mid	6.0±0.2	0.3	994.0±0.2	0.00040±0.000	3.3	6.8
	low	low	4.1±0.4	0.3	995.9±0.4	0.00049±0.000	3.3	5.6
	high	high	3.1±0.3	0.3	996.9±0.3	0.00045±0.000	3.3	6.1
	high	mid	2.7±0.2	0.3	997.3±0.2	0.00036±0.000	3.3	7.6
	high	low	2.3±0.1	0.3	997.7±0.1	0.00026±0.000	3.3	10.5
	mid	high	5.1±0.3	0.3	994.9±0.3	0.00059±0.000	3.3	4.6
	mid	mid	6.2±0.3	0.3	993.8±0.3	0.00047±0.000	3.3	5.8
Switchgrass + N	mid	low	5.2±0.3	0.3	994.8±0.3	0.00042±0.000	3.3	6.5
	low	high	5.4±0.3	0.3	994.6±0.3	0.00049±0.000	3.3	5.6
	low	mid	5.2±0.2	0.3	994.8±0.2	0.00047±0.000	3.3	5.9
	low	low	5.9±0.3	0.3	994.1±0.3	0.00045±0.000	3.3	6.0

Supplementary Table S7a. Eigenvector values for principal component analysis showing relation among soil covariates (excluding soil texture components), principal component 1 (PC1) and principal component 2 (PC2).

Eigenvectors	PC1	PC2
SOC (mg C g ⁻¹ soil)	0.4	0.1
TN (mg N g ⁻¹ soil)	0.4	0.1
Soil pH	0.3	-0.3
Wet aggregate stability (%)	0.3	0.1
POXC (mg C kg ⁻¹ soil)	0.3	-0.3
POXC per unit SOC (mg C g ⁻¹ C)	-0.2	-0.4
Fe _o (mg kg ⁻¹ soil)	-0.05	0.6
Al _o (mg kg ⁻¹ soil)	0.3	0.5
Ca (mg kg ⁻¹ soil)	0.4	-0.1
Mg (mg kg ⁻¹ soil)	0.4	-0.2

Supplementary Table S7b. Loading matrix values for principal component analysis showing relation among soil covariates (excluding soil texture components), principal component 1 (Prin1) and principal component 2 (Prin2).

Loading Matrix	PC1	PC2
SOC (mg C g ⁻¹ soil)	0.96	0.1
TN (mg N g ⁻¹ soil)	0.96	0.15
Soil pH	0.59	-0.46
Wet aggregate stability (%)	0.61	0.12
POXC (mg C kg ⁻¹ soil)	0.72	-0.37
POXC per unit SOC (mg C g ⁻¹ C)	-0.41	-0.59
Fe _o (mg kg ⁻¹ soil)	-0.11	0.84
Al _o (mg kg ⁻¹ soil)	0.57	0.72
Ca (mg kg ⁻¹ soil)	0.90	-0.17
Mg (mg kg ⁻¹ soil)	0.81	-0.21

Supplementary Table S8a. Eigenvector values for principal component analysis with C mineralizability and soil and crop characteristics, principal component 1 (PC1*) and principal component 2 (PC2*).

Eigenvectors	PC1*	PC2*
C mineralizability (mg CO ₂ -C g ⁻¹ C)	-0.26	0.08
SOC (mg C g ⁻¹ soil)	0.38	0.06
TN (mg N g ⁻¹ soil)	0.38	0.1
Soil pH	0.23	-0.26
Wet aggregate stability (%)	0.22	-0.1
Sand (%)	-0.27	0.04
Clay (%)	0.16	0.17
POXC (mg C kg ⁻¹ soil)	0.28	-0.24
POXC per unit SOC (mg C g ⁻¹ C)	-0.16	-0.37
2014 root biomass (g kg ⁻¹ soil)	0.14	-0.09
Above-ground biomass (Mg ha ⁻¹)	-0.14	0.26
Ca (mg kg ⁻¹ soil)	0.36	-0.1
Mg (mg kg ⁻¹ soil)	0.34	-0.18
Fe _o (mg kg ⁻¹ soil)	-0.04	0.55
Al _o (mg kg ⁻¹ soil)	0.23	0.48

Supplementary Table S8b. Loading matrix values for principal component analysis with C mineralizability and soil and crop characteristics, principal component 1 (PC1*) and principal component 2 (PC2*).

Loading matrix	PC1*	PC2*
C mineralizability (mg CO ₂ -C g ⁻¹ C)	-0.64	-0.1
SOC (mg C g ⁻¹ soil)	0.93	0.09
TN (mg N g ⁻¹ soil)	0.95	0.15
Soil pH	0.58	-0.39
Wet aggregate stability (%)	0.53	0.21
Sand (%)	-0.66	0.07
Clay (%)	0.38	0.25
POXC (mg C kg ⁻¹ soil)	0.69	-0.36
POXC per unit SOC (mg C g ⁻¹ C)	-0.4	-0.55
2014 root biomass (g kg ⁻¹ soil)	0.35	-0.13
Above-ground biomass (Mg ha ⁻¹)	-0.34	0.39
Ca (mg kg ⁻¹ soil)	0.9	-0.16
Mg (mg kg ⁻¹ soil)	0.84	-0.26
Fe _o (mg kg ⁻¹ soil)	-0.1	0.8
Al _o (mg kg ⁻¹ soil)	0.58	0.7

Supplementary Table S9. Cumulative C mineralization in incubation experiments at different moisture levels at constant temperature varying between 25° to 35°C.

Study #	Study details	Moisture levels and Cumulative C mineralization	Incubation duration	Study authors
1.	High elevation peatlands of Sierra Nevada, USA.	Greatest at the wettest (-0.1bar) and driest (-4 bar) water potential, U shaped pattern.	392	Arnold <i>et al.</i> 2014
2.	Forest, grassland and cropland soils of Atlantic humid temperate zone, Spain.	40, 60, 80, 100% of field capacity.	42	Guntiñas <i>et al.</i> 2013
3.	Burned and unburned soils of 40-year-old subtropical Chinese Fir forest, China.	Increased with increasing moisture from 25% to 75% WHC.	90	Guo <i>et al.</i> 2012
4.	Soils of arid and semiarid ecosystems on the Mongolian plateau.	Increased 23% from 30 to 60% WFPS and by 176% from 60-90%.	28	Mi <i>et al.</i> 2015
5.	Mediterranean forest soils, Italy	Mineralization increased with increasing moisture content from 20 to 40 to 60 to a maximum at 80% and decreased beyond it, at 100% WHC.	30	Rey <i>et al.</i> 2005
6.	Forest soils of Changbai mountain, (temperate region) Northeast China.	Increased with increasing moisture from 20% to 40% to 60% of mass water content (g g ⁻¹ soil).	42	Qi <i>et al.</i> 2011
7.	Soils of humid mid-subtropical forest soils, South China.	More in mid mass water content (33%) in comparison to low (21%) and high (45%) mass water contents.	45	Wang <i>et al.</i> 2016