Supplementary Material for

Soil organic carbon accrual due to more efficient microbial utilization of plant inputs at greater long-term soil moisture

Itamar A. Shabtai^{a,1,*}, Srabani Das^b, Thiago M. Inagaki^{c,2}, Behrooz Azimzadeh^a, Brian

Richards^d, Carmen Enid Martínez^a, Ingrid Kögel-Knabner^{c,f}, and Johannes Lehmann^{a,e}

*Corresponding author

^aSoil and Crop Sciences, School of Integrative Plant Science, Cornell University, Ithaca,

NY, 14850, USA

^bCenter for Carbon Management and Sequestration, The Ohio State University, Columbus, OH, 43210, USA

^cChair of Soil Science, School of Life Sciences, Technical University of Munich, 85354

Freising, Germany

^dBiological and Environmental Engineering, Cornell University, Ithaca, NY, 14850, USA

^eCornell Atkinson Center for Sustainability, Cornell University, Ithaca, NY, 14850, USA

^fInstitute for Advanced Study, Technical University of Munich, Lichtenbergstraße 2a,

85748 Garching, Germany

¹Current address: Department of Environmental Sciences, The Connecticut Agricultural Experiment Station, New Haven, CT, 06504-1106, USA

²Current address: Department for Environment and Natural Resources, Norwegian Institute for Bioeconomics, Svanvik, Norway

	Moisture level						
Plot	Subplot	[Q1=wettest]	Normalized moisture value				
А	1	Q3	1.037				
	2	Q1	1.387				
	3	Q4	1.034				
	4	Q2	1.196				
	5	Q5	0.936				
F	1	Q5	0.869				
	2	Q3	1.072				
	3	Q5	1.206				
	4	Q4	0.999				
	5	Q2	1.106				
Ι	1	Q4	0.850				
	2	Q3	1.040				
	3	Q2	1.062				
	4	Q1	1.200				
	5	Q5	0.727				
Р	1	Q3	0.990				
	2	Q2	0.990				
	3	Q1	1.224				
	4	Q4	0.930				
	5	Q5	0.805				

Table S1. *Categorical moisture levels and normalized moisture values of the studied soils* (n = 20).

Table S2. Results from elemental and isotope characterization of plant biomass and soil density fractions. The average value is followed by the standard deviation $(\pm S.D.)$ in parentheses. The level of significant from the 2-way ANOVA model of fraction and moisture is reported as *** P < 0.001, or non-significant (ns).

Soil fraction/biomass	C:N	δ ¹³ C (‰)	δ ¹⁵ N (‰)	
Roots	50.21 (7.5)	-28.62 (0.5)	3.50 (1.2)	
Shoots	38.86 (6.1)	-28.11 (0.3)	1.97 (1.2)	
fPOM	29.97 (3.0)	-28.37 (0.5)	4.39 (1.9)	
oPOM	22.88 (2.2)	-28.80 (0.4)	3.81 (1.4)	
MAOM	9.20 (0.19)	-27.35 (0.3)	5.29 (0.7)	
Bulk soil	10.31 (0.4)	-27.53 (0.3)	5.82 (3.2)	
Source of variance				
Fraction	***	***	***	
Moisture ns		***	ns	
Fraction*Moisture	ns	ns	ns	

Table S3. ¹³*C*-*NMR integral ratios and C-NEXAFS peak height ratios of MAOM fractions from composited samples of low, mid, and high moisture levels.*

Moisture level	carboxyl-C/aromatic-C		carboxyl/O-alkyl-C	O-alkyl-C/alkyl-C
	C-NEXAFS	¹³ C-NMR	¹³ C-NMR	¹³ C-NMR
Low	1.349	0.533	0.262	1.454
Mid	1.363	0.594	0.286	1.928
High	1.429	0.615	0.290	2.063

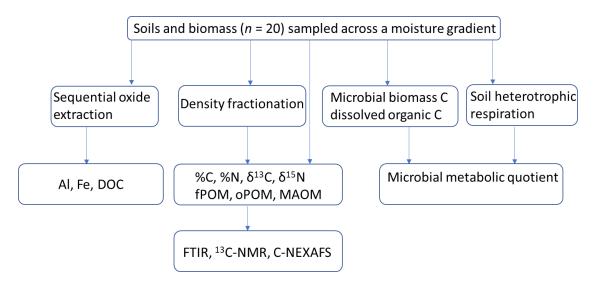


Figure S1. Flow chart of experiments. See Materials and Methods in the main text for details. Soil and biomass from 20 subplots across a long-term soil moisture gradient were sampled. Sequential oxide extraction was performed and Al, Fe, and C concentrations in sodium pyrophosphate (PY), hydroxylamine hydrochloride (HH), and sodium dithionite (DIT) extractions were measured. Density fractionation was done to isolate the free particulate organic matter fraction (fPOM), occluded particulate organic matter fraction (oPOM), and mineral associated organic matter fraction (MAOM). C and N elemental and isotopic analyses was done on soil fractions, bulk soils, and plant biomass. Fourier Transform Infrared (FTIR) spectroscopy, C Near Edge X-ray Absorption Fine Structure (NEXAFS) spectroscopy, and ¹³C Nuclear Magnetic Resonance (NMR) spectroscopy was used to characterize the chemical composition of the fractions and bulk soils. Soil microbial biomass and heterotrophic respiration were measured and used to calculate the microbial metabolic quotient.

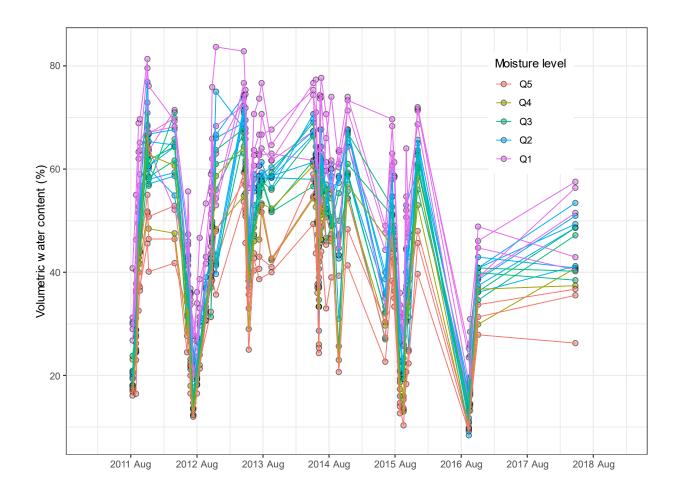


Figure S2. Soil volumetric water content from 2011 to 2018. Each data point represents a mean of time domain reflectometry (TDR) measurements (n = 3). Data from Das et al. (2019)

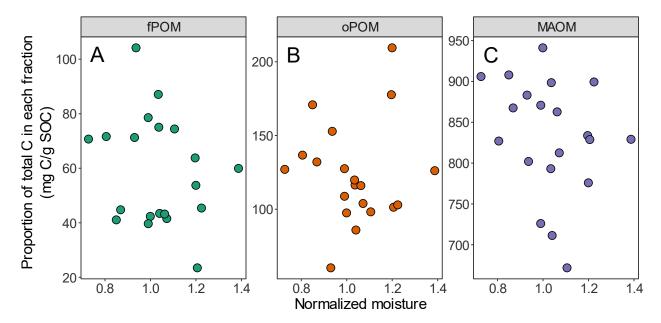


Figure S3. Proportion of total carbon (*C*) in free particulate (*A*), occluded particulate (*B*), and mineral associated organic matter fractions (*C*)((*fPOM*, oPOM, and MAOM, respectively), relative to total SOC, as a function of normalized soil moisture values. Linear regression models were non-significant.

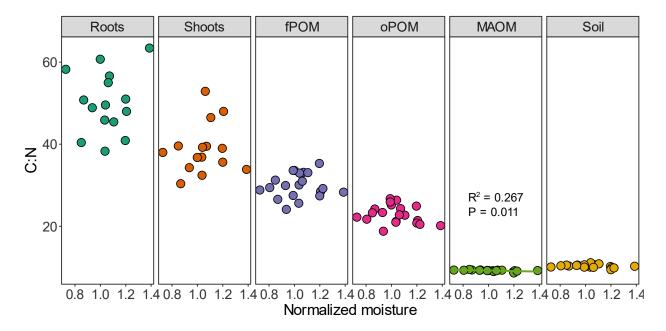


Figure S4. C:N ratio of plant biomass, and free particulate, occluded particulate, and mineral associated organic matter fractions ((fPOM, oPOM, and MAOM, respectively) as function of normalized soil moisture values. Significant regression models are shown with regression lines, regression coefficients, and P values.

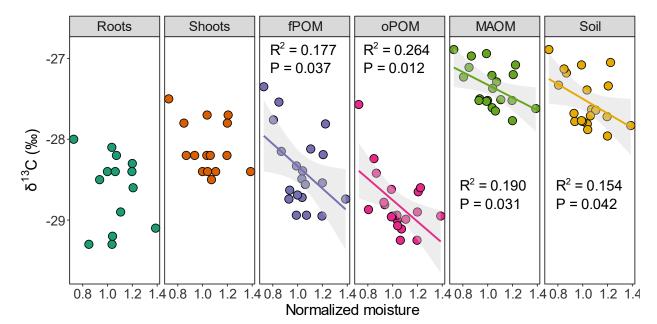


Figure S5. δ^{13} C values of plant biomass, and free particulate, occluded particulate, and mineral associated organic matter fractions ((fPOM, oPOM, and MAOM, respectively) as function of normalized soil moisture values. Significant regression models are shown with regression lines, regression coefficients, and P values.

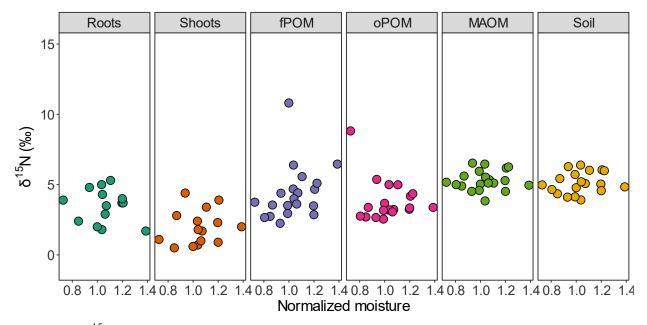


Figure S6. δ^{15} N values of plant biomass, and free particulate, occluded particulate, and mineral associated organic matter fractions ((fPOM, oPOM, and MAOM, respectively) as function of normalized soil moisture values. Significant regression models are shown with regression lines, regression coefficients, and P values.

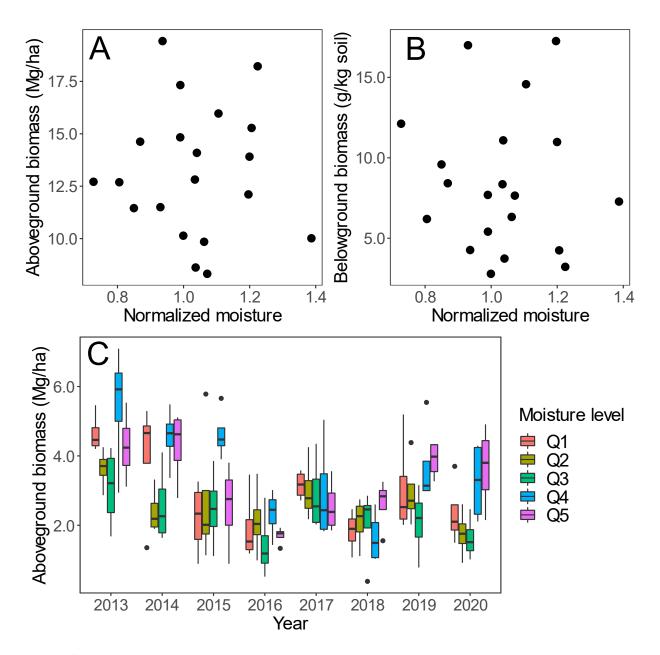


Figure S7. *Cumulative aboveground (A) and belowground (B) biomass sampled in 2013-2015 as a function of normalized moisture values, and annual above ground biomass sampled in 2013-2020 (C). Linear Regression models for data plotted in A and B were non-significant.*

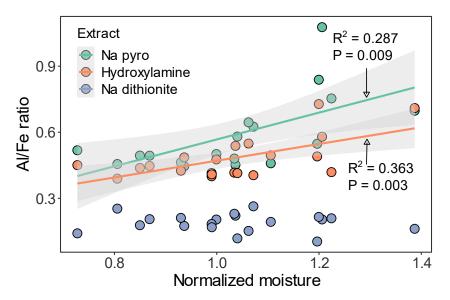


Figure S8. Al/Fe ratio in different oxide extractions as a function of normalized soil moisture values. Significant regression models are shown with regression lines, regression coefficients, and P values.

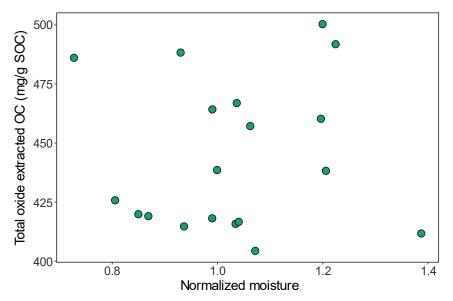


Figure S9. Total organic carbon (OC) in pyrophosphate, hydroxylamine, and dithionite extracted oxide phases, normalized to soil organic carbon (SOC) content as a function of normalized soil moisture values. Regression model was non-significant.

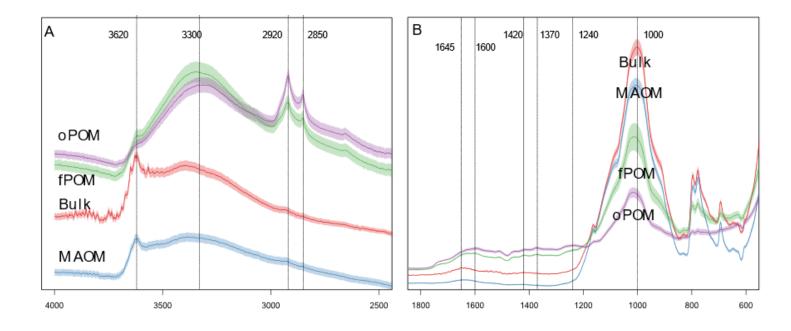


Figure S10. ATR-FTIR spectra in the range of $2500 - 40000 \text{ cm}^{-1}$ (A) and in the range of $550 - 1840 \text{ cm}^{-1}$ (B) of bulk soils and MAOM fractions (n = 20) and fPOM and oPOM fractions (n = 3) averaged across all long-term normalized moisture values.

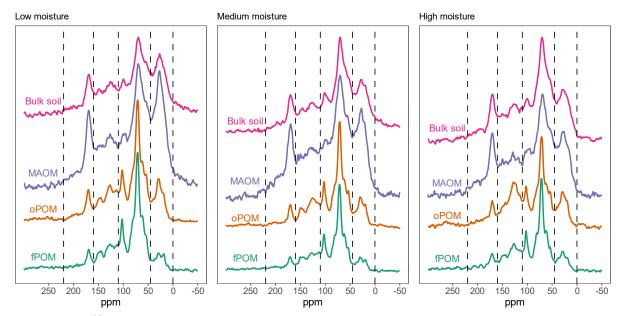


Figure S11. ¹³*C NMR spectra of fPOM, oPOM, MAOM, and bulk soils from composited sampled from low, mid, and high moisture levels.*

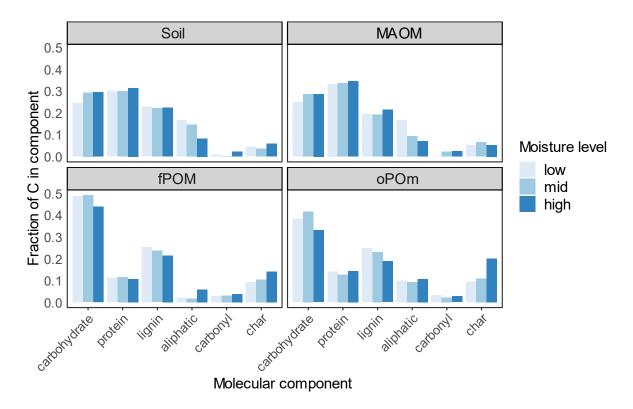


Figure S12. Molecular mixing model results for ${}^{13}C$ -NMR analysis. Relative amount of C in different molecular components for each soil fraction and normalized moisture level.

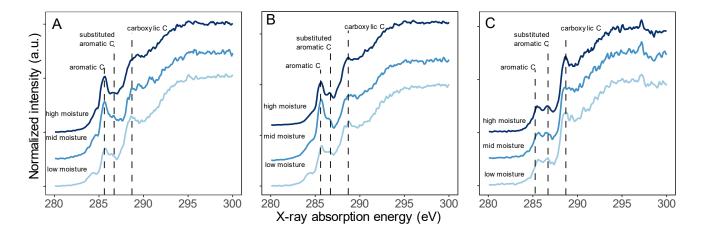


Figure S13. *C K-edge NEXAFS spectra of fPOM (A), oPOM (B), and MAOM (C) fractions from low, mid, and high moisture levels.*

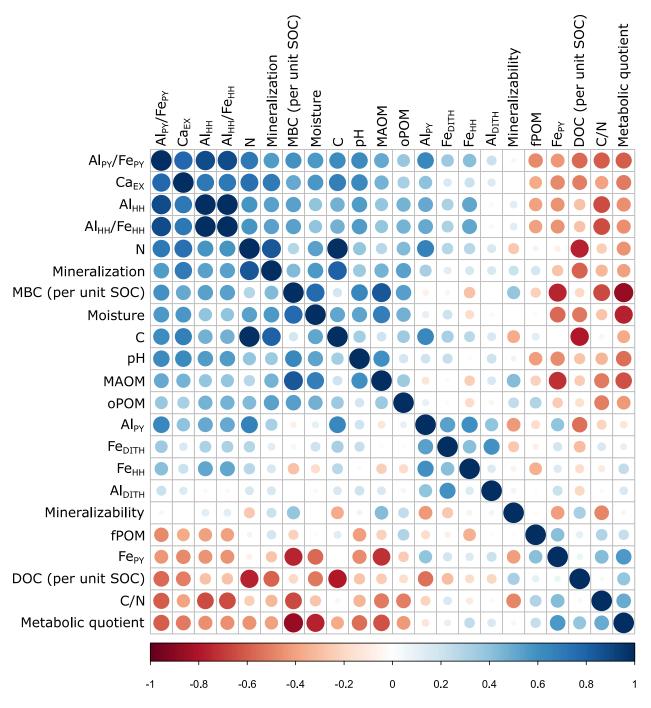


Figure S14. Heatmap of Pearson correlations (r).

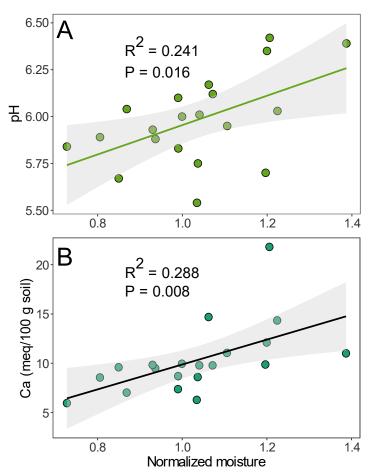


Figure S15. Soil *pH*(*A*) and exchangeable Ca (*B*) as function of normalized soil moisture value. Significant regression models are shown with regression lines, regression coefficients, and *P* values.

Statistical analyses additional information:

We constructed the following partial least squares (PLS) regression models for SOC, mineralization, and mineralizability, with the following explanatory variables

SOC ~ Microbial biomass C, Ca_{EX}, pH, Al_{PY}, Fe_{PY}, Al_{HH}, Fe_{HH}, Al_{DITH}, Fe_{DITH}, Al_{PY}/Fe_{PY}, Al_{HH}/Fe_{HH}, Al_{DITH}/Fe_{DITH}, normalized moisture value, relative amount of oPOM, fPOM, and MAOM, and microbial metabolic quotient

Mineralization ~ %C, soil C:N, DOC, Microbial biomass C, Ca_{EX}, pH, Al_{PY}, Fe_{PY}, Al_{HH}, Fe_{HH}, Al_{DITH}, Fe_{DITH}, Al_{PY}/Fe_{PY}, Al_{HH}/Fe_{HH}, Al_{DITH}/Fe_{DITH}, normalized moisture value, relative amount of oPOM, fPOM, and MAOM, and microbial metabolic quotient

Mineralizability ~ %C, soil C:N, DOC, Microbial biomass C, Ca_{EX}, pH, Al_{PY}, Fe_{PY}, Al_{HH}, Fe_{HH}, Al_{DITH}, Fe_{DITH}, Al_{PY}/Fe_{PY}, Al_{HH}/Fe_{HH}, Al_{DITH}/Fe_{DITH}, normalized moisture value, relative amount of oPOM, fPOM, and MAOM, and microbial metabolic quotient