Supplementary material

Simulated Soil Respiration is Sensitive to Soil Hydraulic Properties from Intact vs. Repacked Cores

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1. Soil hydraulic properties measurements:

Soil water retention curves (SWRC) play an important role in understanding carbon cycling and are crucial to understanding soil water retention behavior that affects drainage, nutrient transport, and microbial activity. High-quality standardized pore scale data on soil structure inferred from the SWRC can be used to refine existing models to better represent field conditions as land use and environmental change occur in real time. How different soils behave while inundated, dry, or partially saturated is measured using soil water retention curves (SWRC).

1.1. Site characteristics and Soil Collection

Soil sample collection followed a standardized kit and field sampling protocol developed by MONet, utilizing custom AMS 7.62 × 30 cm coring barrels (M404.74) and liners (SKU 422.02). All samples were shipped overnight on blue ice, maintaining their original field orientation. Texture analysis by hydrometer, performed by Regen Ag LLC, identified four cores representing a range of soil textures: sandy loam, silt loam, loam, and silty clay. The sandy loam core was collected from Harvard Forest, Massachusetts (42.5365°N, -72.1760°W), a northern hardwood and coniferous forest with a mean annual temperature of 7.4°C and 1080 mm of precipitation. The silt loam core came from Steele Creek, Alaska (64.9146°N, -147.4858°W), a mixed hardwood–coniferous forest with a mean annual temperature of -1.94°C and 359.9 mm of precipitation. The loam core was obtained from Crested Butte, Colorado (38.8536°N, -107.0663°W), an alpine hardwood forest with a mean annual temperature of 0.1°C and 626 mm of precipitation. The silty clay core was collected from The Morton Arboretum, Illinois (41.815216, -88.084200), a hardwood and coniferous forest with a mean annual temperature of 9.3°C and 978.4 mm of precipitation.

Table S1 Site-specific information for locations where soil samples were collected.

Texture	Site	Latitude	Longitude	Dominant Vegetation	Mean Annual Precipitation	Mean Annual Temperature
Sandy Loam	Harvard Forest, MA	42.5365	-72.1760	Northern hardwood, coniferous forest	1080 mm (42.5 in.)	7.4°C (45°F)
Silt Loam	Steele Creek, AK	64.9146	-147.4858	Hardwoods, Coniferous	359.9 mm (14.17 in.)	-1.94°C (28.5°F)

				Forest		
Loam	Crested Butte, CO	38.8536	-107.0663	Hardwoods, Alpine forest	626 mm (24.6 in.)	0.1°C (32.2°F)
Silty Clay	The Morton Arboretum, IL	41.815216	-88.084200	Hardwoods, Coniferous forest	978.4 mm (38.52 in.)	9.3°C (48.8°F)

1.2. Preparation of HYPROP 2 Unit

HYPROP instruments are provided with detailed instructions and auxiliary equipment that facilitate their use. Soil core samples are instrumented with two tensiometers – one short and one long – that are installed from the bottom of the core. Sensor units and tensiometer shafts were degassed using the HYPROP refill unit. The entire unit consists of four separate but connected instruments: a vacuum pump, vacuum mount, beaker mount, and HYPROP 2 sensor (Figure 2). All components are connected by tubing and sealed to prevent air leaks. Each sensor unit was filled with Milli-Q water before degassing. All tensiometer shafts and sensor units were degassed for minimum of 24 hours.

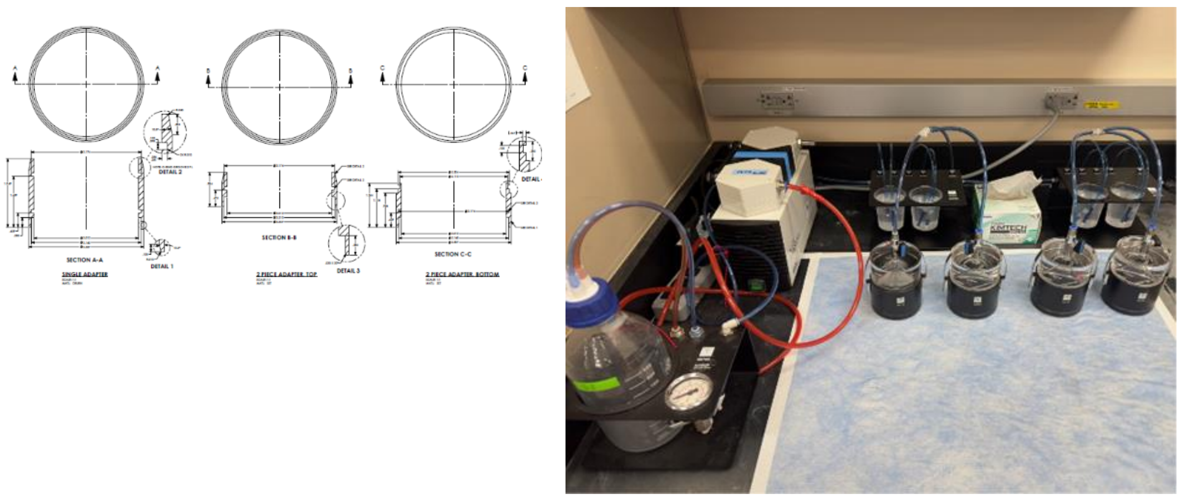

Figure S1 Schematic representation of custom HYPROP unit used for measuring water retention curve, and degassing station for sensor heads and tensiometer units.

1.3. Preparation and analysis of Intact and repacked soil cores

For each soil sample, soil water retention curves (SWRC) were generated utilizing a HYPROP 2 (Meter Group). SWRC for each soil type was measured twice: one from intact core and then again after sieving, homogenization, and repacking the soil to match its original dry mass bulk density. Intact measurements were completed using a custom adaptor ring (SI Figure S1). Soil from the intact column was oven-dried and sieved to 4 mm to eliminate large rocks or debris. It was then repacked to the original dry bulk density within the same custom adaptor ring/core liner assembly used for the intact core. Repacking was carried out in four lifts, each 1.25 cm, to minimize over compaction at the top or bottom of the sample ring. Both intact and repacked soils were saturated in a 0.01 M CaCl2 solution. Afterwards, the saturated hydraulic conductivity (K_s) of each sample was determined using Meter Group's falling head KSAT. Van

Genuchten parameters (α, n, θ_r, and θ_s) were then calculated using SoilView Analysis Software from METER Group. These parameters were cross-referenced with data from the HiHydroSoil v2.0 database in order to compare direct measurements of soil hydraulics to publicly available data that is often used to parameterize soil C models.

1.4. HYPROP 2 Data analysis

The SWRC is a function, and most commonly interpreted using the van Genuchten equation:

$$\theta_{eff} = \frac{\theta - \theta_r}{\theta_s - \theta_r} = \left[\frac{1}{1 + (\alpha\psi)^n}\right]^{1-\frac{1}{n}}, \tag{S1}$$

where θ_{eff} is effective saturation, θ_s is saturated water content, θ_r is residual water content, α is related inversely to the air entry suction during drying, and n denotes the slope of the SWRC as influenced by pore size distribution. HiHydroSoil v2.0 provide high resolution soil maps of Van Genuchten model parameters θ_s θ_r, α, n, and saturated hydraulic conductivity (K_s).

Table S2 van Genuchten water retention curve parameters, saturated hydraulic conductivity (K_s), dry density, soil texture measured in lab (white) compared to HiHydroSoil v 2.0 prediction (grey).

Texture	Sample	KSAT cm/day	θr	θs	Alpha	n	Sand (%)	Silt (%)	Clay (%)
Silt Loam	Intact	52.0	0.034	0.786	0.0108	1.218	24.8	60.4	14.8
	Repacked	82.0	0.014	0.539	0.0101	1.615			
	HiHydroSoil v 2.0	6.9	0.041	0.633	0.0081	1.347			
Loam	Intact	136.0	0.049	0.692	0.0226	1.203	37	39.8	23.2
	Repacked	52.0	0.079	0.652	0.0131	1.404			
	HiHydroSoil v 2.0	9.9	0.041	0.508	0.0154	1.319			
Silty Clay	Intact	9.9	0.132	0.514	0.0207	1.143	15.4	40.8	43.8
	Repacked	13.0	0.053	0.424	0.0281	1.192			
	HiHydroSoil v 2.0	4.5	0.041	0.434	0.0064	1.214			
Sandy Loam	Intact	287.6	0.043	0.52	0.0199	1.196	62.4	25.8	11.9
	Repacked	112.0	0.076	0.5	0.0099	1.389			
	HiHydroSoil v 2.0	19.2	0.041	0.501	0.0284	1.391			

2. Modeling Framework

To evaluate how uncertainties in soil hydraulic properties and limitations in process representation influence carbon (C) flux simulations, we used the Millennial soil C cycle model (Abramoff et al., 2022).

The default moisture modifier in Millennial does not account for soil structural heterogeneity, which affects the spatial colocation of soil organic carbon (SOC) and microbes. Empirical studies have shown that higher colocation enhances microbial activity (Chakrawal et al., 2020; Shi et al., 2021).

Yan et al. (2018) addressed this limitation by proposing a moisture modifier that incorporates a *colocation factor*, parameterized as a function of clay content. High clay content can occlude SOC, reducing its microbial accessibility and activity, particularly under drier conditions (see Figure S2 and S3). In this formulation, the strength of microbial activity suppression increases with clay content, capturing the influence of structural heterogeneity.

We hypothesized that the slope of the linear relationship between the colocation factor and clay content reflects the degree of soil structural constraint, with intact soils expected to exhibit greater SOC inaccessibility than repacked soils. By varying this slope, we simulated the effects of structural heterogeneity on microbial activity and systematically examined its impact on SOC fluxes across different textures. Specifically, we compared annual respiration rates and steady-state SOC stocks using the original *Millennial v2* moisture modifier and the function proposed by Yan et al. (2018).

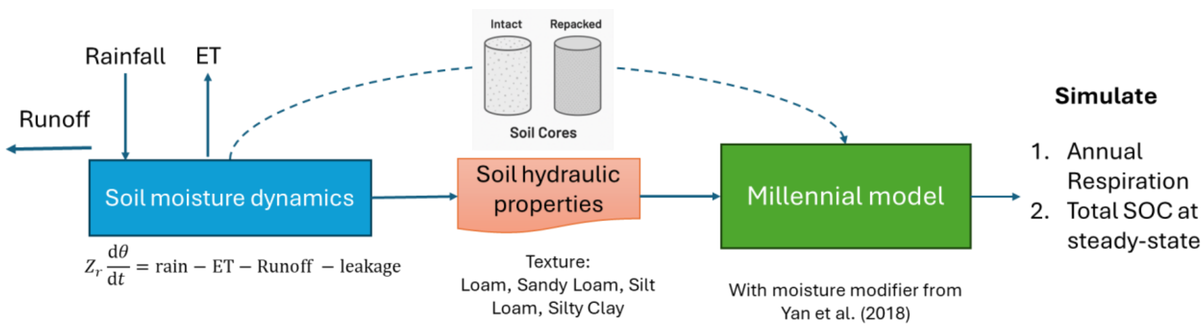

Figure S2 Schematic of modeling framework.

2.1. Model Setup

Simulations were conducted for the top 30 cm of soil using Millennial, operating at a daily time step and driven by daily temperature, plant C input, and soil moisture (Figures S4 and S5). Temperature and net primary production (NPP) were generated following the approach of the MEMS v1 model (Robertson et al., 2019). Daily temperature followed a sinusoidal pattern bounded by prescribed minimum and maximum values for a Northern Hemisphere climate. Plant C input was estimated by offsetting daily NPP to reflect seasonal litterfall, assuming a fixed fraction of NPP enters the soil as litter.

Model parameters were adopted from Abramoff et al. (2022). For all simulations, external forcings were held constant while soil hydraulic properties were varied (see Table S2). To simulate moisture dynamics, we developed a soil water balance module coupled to *Millennial*, resolving daily soil moisture in the top 30 cm based on hydraulic parameters.

2.2. Implementing modified moisture function in Millennial

To incorporate the effects of soil microscale heterogeneity on microbial activity, we modified the moisture sensitivity functions governing particulate organic matter (POM) depolymerization and dissolved organic matter (DOM) uptake. Notably, Millennial v2 uses the same moisture modifier for POM depolymerization to represent moisture effects on sorption, and on aggregate disruption and formation. Consequently, all these fluxes were influenced by our modified function.

For POM depolymerization, we introduced an exponent '*a*' to the standard moisture modifier (s_{wd}), resulting in the modified expression:$s_{wd} = \left(\frac{\theta}{\phi}\right)^{0.5a}$ This formulation extends the default function in Millennial v2, where $s_{wd} = \left(\frac{\theta}{\phi}\right)^{0.5}$, allowing for the modulation of moisture sensitivity based on soil structural properties.

For DOM uptake, we adopted the moisture modifier proposed by Yan et al. (2018), given by:

$$s_{wb} = \begin{cases} \frac{K_\theta + \theta_{op}}{K_\theta + \theta}\left(\frac{\theta}{\theta_{op}}\right)^{1 + am}, \theta < \theta_{op} \\ \left(\frac{\phi - \theta}{\phi - \theta_{op}}\right)^{b}, \theta \geq \theta_{op} \end{cases}, \tag{S2}$$

where θ is soil moisture, ϕ is porosity, θ_{op} is optimal moisture at which microbial activity is maximum, K_θ is a moisture constant, a: colocation factor given as a function of clay % ($a = 2.8 * clay\%\ for\ clay\% < 40, otherwise\ a = 1$), $m = 1 - \frac{1}{n}$ is the saturation exponent, and b is the oxygen supply restriction factor. Detailed description of these parameters are available from Yan et al. (2018). This expression replaces the default Millennial v2 moisture modifier used for DOM uptake fluxes. Parameter values were implemented as recommended in Table 1 of Yan et al. (2018). A comparison between the original and modified moisture functions is provided in Figure S3.

We further assumed that the relationship between the colocation factor and clay content described by Yan et al. (2018) characterizes intact soils, particularly the linear slope between '*a*' and clay percentage (see Figure S2). The slope of this relationship modulates the degree to which soil structural heterogeneity constrains microbial access to substrates. For instance, in intact soils, increasing clay content may more strongly restrict soil organic carbon (SOC) accessibility than in repacked soils. Accordingly, we varied the slope of the colocation factor–clay relationship to simulate structural heterogeneity effects on microbial activity, enabling a systematic evaluation of how intact versus repacked soil cores influence SOC fluxes across soil textures. We tested a range of values for '*a*' to assess the implications for model predictions in repacked soils. Lastly, we treated soil hydraulic properties estimated from HiHydroSoil v2 database the same as intact samples.

2.3. Soil moisture dynamics

Soil moisture dynamics was simulated at the daily time step following a simplified moisture balance assuming a homogeneous soil of 30cm thickness with uniform hydraulic properties that vary based on soil texture and measurement methods such as intact vs repacked cores. The mass balance is given by,

$$Z_r \frac{\mathrm{d}\theta}{\mathrm{d}t} = \mathrm{rain} - \mathrm{ET} - \mathrm{Runoff} - \mathrm{leakage}, \tag{S3}$$

where, θ is soil moisture in unit m^3/m^3, rain, evapotranspiration (ET), runoff and leakage are in m/day, and Z_r is the soil depth equal to 30 cm. For the transient simulation, rainfall for year 2020 from Harvard

Forest & Quabbin Watershed site from NEON data product DP1.00006.001 NEON (2025), and ET was simulated as function of soil moisture. Runoff is the saturation excess surface runoff, and leakage is vertical drainage of water assumed equal to the hydraulic conductivity.

Table S3 Moisture modifier function for particulate and dissolved organic matter uptake from Millennial v2 and Yan et al. (2018)

<table>
<tr><td colspan="2">Moisture modifier for POM uptake</td></tr>
<tr><td>Yan et al 2018</td><td>$s_{wd} = \left(\frac{\theta}{\phi}\right)^{0.5a}$</td></tr>
<tr><td colspan="2">Moisture modifier for POM uptake</td></tr>
<tr><td>Yan et al 2018</td><td>$s_{wb} = \begin{cases} \frac{K_\theta + \theta_{op}}{K_\theta + \theta} \left(\frac{\theta}{\theta_{op}}\right)^{1+a\,n_s}, \theta < \theta_{op} \\ \left(\frac{\phi-\theta}{\phi-\theta_{op}}\right)^{b}, \theta \geq \theta_{op} \end{cases}$</td></tr>
<tr><td colspan="2">θ: soil moisture,
ϕ: porosity,
a: colocation factor given as a function of clay % ($a = \frac{2.8}{clay\%}\ if\ clay\% < 40\ else\ 1$), (Yan et al., 2018)
θ_{op}: optimal moisture at which microbial activity is maximum, (Yan et al., 2018)
λ: Dependence of rate on matric potential (Abramoff et al., 2022)
$k_{a,min}$: Minimum relative rate in saturated soil (Abramoff et al., 2022)</td></tr>
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Table S4. Model parameters from the moisture sensitivity function from Yan et al. (2018).

Soil texture	**Core type**	$\boldsymbol{n}$	$\boldsymbol{m}$	$\boldsymbol{\theta_{op}/\theta_s}$	$\boldsymbol{b}$
Silt Loam	Intact	1.218	0.178982	0.8	0.98
	Repacked	1.615	0.380805	0.8	0.98
	HiHydroSoil v2.0	1.347	0.25761	0.8	0.98
Loam	Intact	1.203	0.168745	0.68	0.8
	Repacked	1.404	0.287749	0.68	0.8
	HiHydroSoil v2.0	1.319	0.24185	0.68	0.8
Silty Clay	Intact	1.143	0.125109	0.6	0.75
	Repacked	1.192	0.161074	0.6	0.75
	HiHydroSoil v2.0	1.214	0.176277	0.6	0.75
Sandy Loam	Intact	1.196	0.16388	0.7	0.74
	Repacked	1.389	0.280058	0.7	0.74
	HiHydroSoil v2.0	1.391	0.281093	0.7	0.74

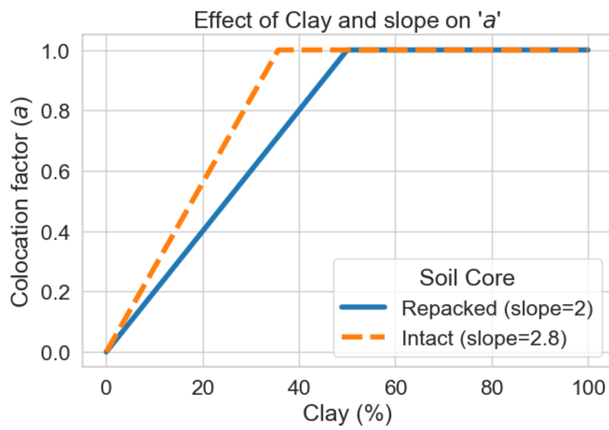

Figure S3 Linear relationship between colocation factor (a) and clay content with varying slope.

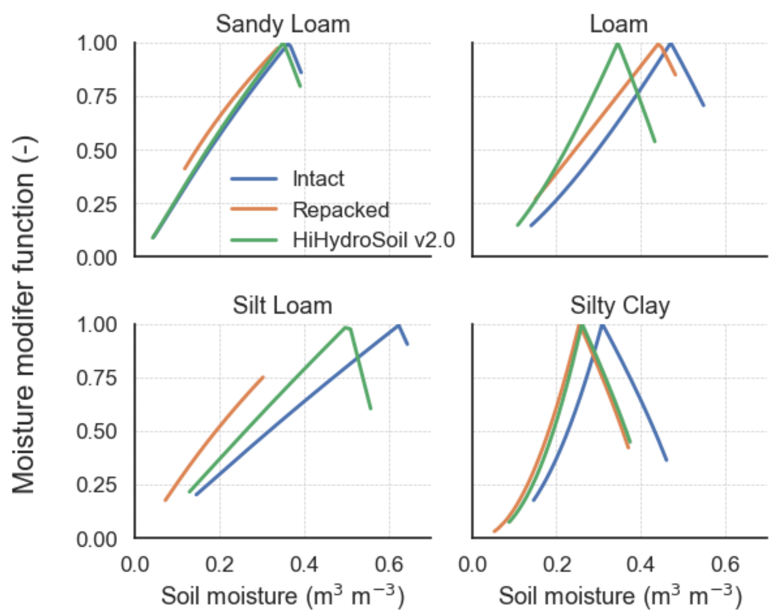

Figure S4 Moisture sensitivity factor as a function of soil moisture using modified moisture function in Millennial model. Note that the range of soil moisture is the same as simulated from Figure S7.

2.4. Model Scenarios

We conducted both steady-state and transient simulations to evaluate how variations in soil hydraulic properties—derived from intact cores, repacked soils, and the global HiHydroSoil v2.0) database—affect modeled carbon (C) dynamics.

2.4.1. Steady-State Simulations:

These simulations were used to compare total soil C stocks under equilibrium conditions as a function of soil hydraulic properties. We assumed a colocation factor of a=2.8 for intact and HiHydroSoil -based scenarios, consistent with Yan et al. (2018). For repacked soils, we reduced this value to a=2, reflecting reduced soil structural complexity. All other soil hydraulic parameters (e.g., water retention curve parameters) were varied according to observed or estimated values specific to each scenario.

2.4.2. Transient Simulations:

In the transient simulations, we simulated one year of soil C dynamics for all soil textures using the modified version incorporating structural heterogeneity (Yan et al., 2018) (Figure SXX). This allowed comparison of the daily dynamics of heterotrophic respiration rates under different representations of moisture sensitivity.

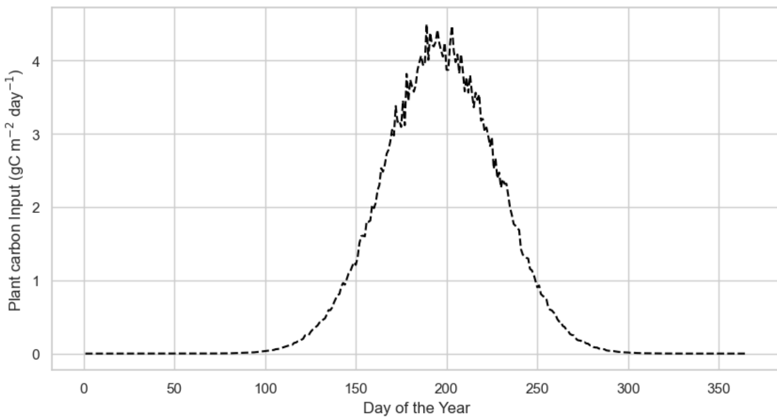

Figure S5 Simulated plant C input to Millennial model

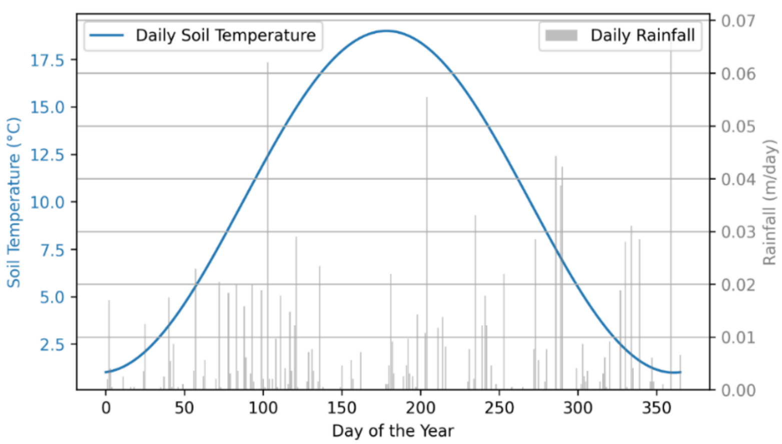

Figure S6 Simulated daily temperature and observed rainfall obtained from HARV NEON site.

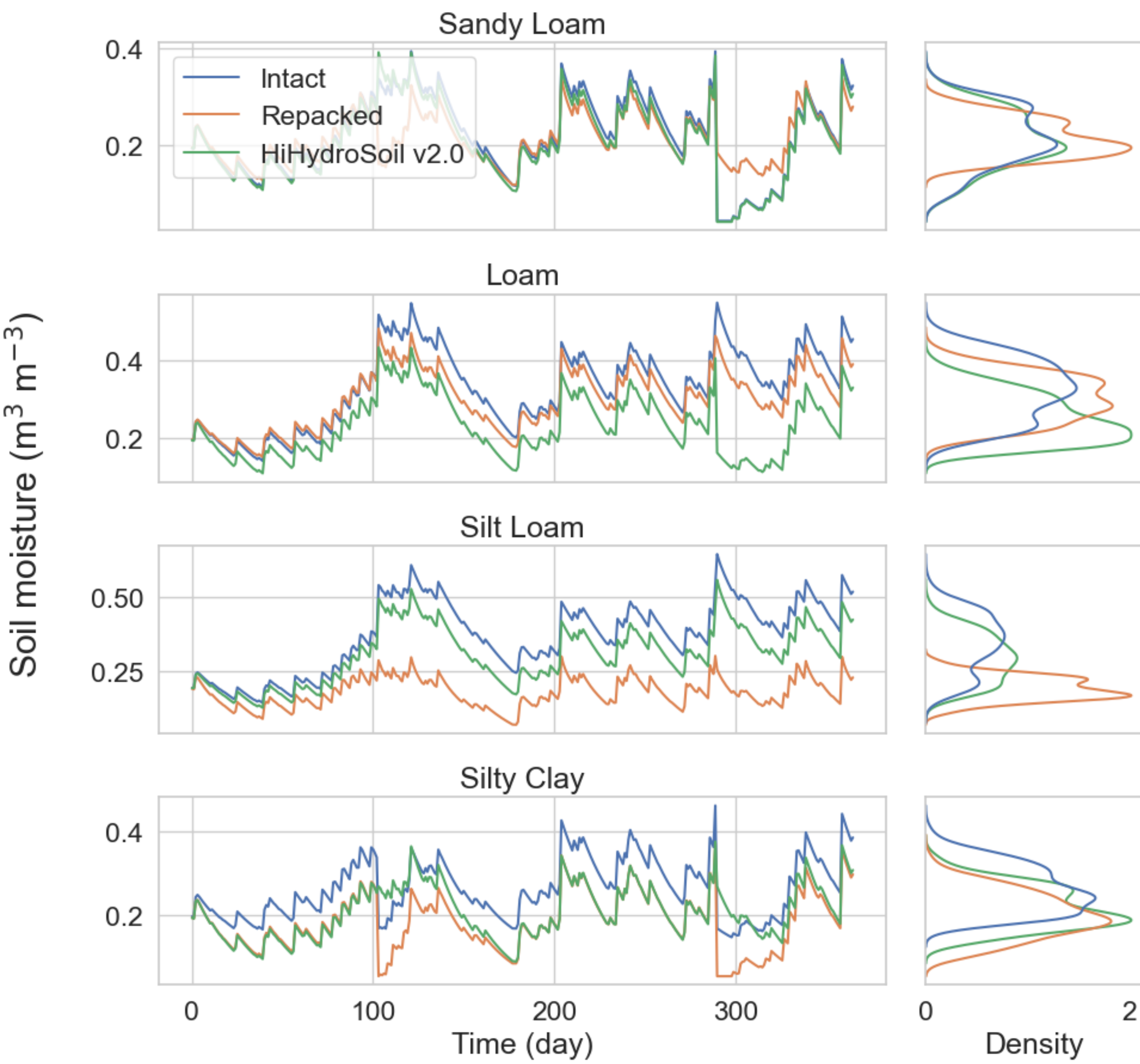

Figure S7 Simulated soil moisture for different soil texture and measurement sources.

Abramoff, R.Z., Guenet, B., Zhang, H., Georgiou, K., Xu, X., Viscarra Rossel, R.A., Yuan, W., Ciais, P., 2022. Improved global-scale predictions of soil carbon stocks with Millennial Version 2. Soil Biol. Biochem. 164, 108466. https://doi.org/10.1016/j.soilbio.2021.108466

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