Improving Predictions of Potential Soil Respiration Across the Continental United States with Soil Organic Matter Composition

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Abstract

Despite the importance of microbial respiration of soil organic matter (SOM) in regulating carbon flux between soils and the atmosphere, soil carbon (C) cycling models remain primarily based on climate and soil properties, leading to large uncertainty in their predictions. Molecular data have long been proposed as a promising avenue for resolving modeling errors, but evidence for improved predictions of soil C cycles with high-resolution measurements remains mixed. With data from the 1000 Soils Pilot of the Molecular Observation Network (MONet), we analyzed the molecular composition of water-extractable SOM from 66 soil cores across the United States to address this knowledge gap. Our innovation lies in using machine learning (ML) to distill the thousands of SOM formula that we detected per sample into tractable units. Then, we compared ML predictions of measured potential soil respiration using (1) a suite of standard soil physicochemical data, (2) ultrahigh-resolution SOM composition independently, and (3) in combination with physicochemistry to assess the added value of molecular information to predict soil respiration. In surface soils (0-10 cm), water-extractable SOM chemistry alone provided better estimates of potential soil respiration than soil physicochemical factors alone, and using the combined sets of predictors yielded the highest explanatory power of soil respiration rates. In contrast, in subsoils (>10 cm), SOM composition did not improve ML-based respiration model performance, possibly due to the greater importance of mineral-associated SOM below the surface layer. Our results underscore the potential of integrating SOM composition into carbon cycle modeling for enhanced predictions of terrestrial-atmosphere climate feedback.

Introduction

Soil respiration is estimated to release 60-100 Gt of C to the atmosphere per year,[1, 2] six to ten times as much C as released by fossil fuel combustion (~10 Gt C[3]). Microbial respiration of soil organic matter (SOM) is one of the most important contributors to soil carbon dioxide (CO_2) emissions and a critical link in the global C cycle.[4] With increasing temperatures under climate change, soil C repositories are vulnerable to increased rates of microbial respiration,[5-7] which can lead to positive feedbacks in global CO_2 emissions and temperature rises.[8] Despite decades of research, soil C fluxes remain one of the largest uncertainties in global climate predictions.[9-14] Novel molecular measurements have recently been applied to identify SOM composition in an effort to understand molecular-scale processes that could improve model predictions of CO_2 fluxes.[15-18] Despite these efforts, our attempts to improve soil C model predictions by refining chemical pools have yielded mixed results.[19-21]

The interplay of factors such as soil moisture, pH, nutrients, mineralogy, and SOM concentration and chemistry governs microbially-derived transformations of SOM;[22-27] but these relationships are difficult to constrain.[4, 28] The most commonly used modeling approaches are based on Raich’s model, which estimates respiration primarily as a function of temperature and water availability.[29], [30] Newer process-based model formulations use an additional suite of physical and biogeochemical measurements to represent microbial and mineral processes. They incorporate SOM chemistry either through several discrete pools or through their thermodynamic properties.[21, 31-34] With large spatiotemporal heterogeneity and limited availability of comprehensive and standardized measurements at regional-to-continental scales, accurate predictions of microbial SOM decomposition across different ecosystems remain challenging.[35]

A better understanding of SOM concentration, composition, and bioavailability may enhance our ability to predict soil C cycling processes through their controls on soil respiration and related enzymatic activities.[21, 31-34] Variations in the bioavailability of chemical classes of SOM are mediated by geochemical conditions and biophysical constraints, such as microbial biomass and necromass, reactive metals and minerals, organic and mineral horizon thickness, and other climate-related variables.[36] For example, coarse-textured soil is more conducive to decomposition of chemically labile litter-derived C potentially due to higher fungal activity in organic-rich horizons.[37, 38] In addition, the interface between fresh litter inputs and soil minerals can serve as a hotspot for microbial breakdown of C found in the litter, resulting in the formation of soil aggregates and organo-mineral associations.[39] This variability underlines the essential need to identify unique subsets of SOM formula that contribute more to soil respiration among different ecosystems and soil depths.

The distillation of multidimensional SOM composition profiles into a tractable set of formula that influence soil respiration is a key challenge in soil ecology.[15, 28, 40-45] Unsupervised machine learning models that summarize large data into a small number of significant features have been widely used to study microbial communities, SOM composition, and other environmental problems with multidimensional data.[46] Dimensionality reduction such as Principal Component Analysis (PCA)[47-49] and clustering methods such as hierarchical clustering analysis[50-52] are the most common tools to explore large molecular datasets. Although these tools are beginning to be applied to determine the relationship between SOM composition and soil physicochemistry,[49] it is still challenging to extract a subset of SOM substrates associated with specific processes, like soil respiration.

Although ultrahigh mass resolution measurements can provide unprecedented characterization of the thousands of individual formulae that comprise SOM, the interpretation of these data types largely remains guided by coarse chemical and ecological groupings. Here, we develop models using semi-supervised machine learning (non-negative matrix factorization with custom *k*-means clustering, NMF*k*) to reduce the complexity of molecular information into *k* distinct signatures of water-extractable SOM chemistry at two depths in cores collected across the continental United States. We then explore the extent to which these signatures and NMF*k*-enabled feature set can provide additional insight into rates of soil respiration beyond variables that are more routinely collected. By examining a multitude of physicochemical and SOM signatures, our goal is to elucidate the specific subset of SOM substrates that may be vital to understanding and predicting below-ground C storage.

Methods

Soil sampling and characterization.

As part of the 1000 Soils Pilot study for the Molecular Observation Network (MONet) program, we collected 66 soils from across the continental US using standardized sampling procedures described by Bowman et al.[53] (Figure S1). Two long cores (30 cm) and three short cores (10 cm) were collected at each site. We also conducted field measurements, including soil temperature, volumetric water content, vegetation type, and weather conditions. Cores were shipped on ice overnight to the Pacific Northwest Laboratory for further analysis. A full description of sampling and analytical methodologies is available in Supporting Information and Bowman et al.[53]

Water extractable SOM characterization.

We extracted water-soluble SOM from soils using solid phase extraction and analyzed using a Bruker 7-T Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) at the Environmental Molecular Sciences Laboratory (EMSL) in Richland, WA. More details on SOM extraction methods and FTICR MS analysis are in Supporting Information and Bowman et al.[53]

Raw FTICR MS data was processed with CoreMS (Python package, installed on 2022/11/22),[54] including signal processing, peak detection, and molecular formula assignment. Noise thresholding was performed with signal-to-noise threshold (5 std.), mass error (0.3 ppm), and stoichiometric limits from domain knowledge (supporting information). Suwannee River fulvic acid (SRFA) standards were used to set a calibration threshold for all soils in the same batch. Molecular formula was assigned based on both accurate mass and filtered by their confidence score from CoreMS. After calibration and formulae assignment, we filtered the assigned peaks by m/z between 200 to 1,000, present in at least 2 out of 3 replicates, not present in two or more lab blanks, and with formulae confidence scores (combines m/z error and isotopic pattern)[54] above 0.7. We predicted compound classes of the filtered formulae based on O/C and H/C ratios of van Krevelen classes.[55, 56] The suffix "-like" in chemical classes indicates the uncertainty of the van Krevelen classification method.[56] We converted the peak intensity values to present/absent (1/0) and separated the final dataset by soil depth (surface vs. subsoil) for statistical analysis. Alpha diversity was calculated as the total number of SOM formulae identified in each sample.

Data analysis and machine learning methods.

We used linear regression models to evaluate the relationship between soil potential respiration and soil physicochemical variables. To avoid the impacts of different magnitudes of the data that might lead to biased relationships, we performed $\log_{10}$ transformation on potential respiration rates, total C, total N, total sulfur, and Mn concentration. *stats.linregress* function from *scipy* package (v 1.11.4) in Python (v 3.7.1) was applied to calculate the fitted line, r^2 value ($rvalue^2$, Pearson correlation), and p-value (*pvalue*). Pairwise plots with regression fitting were generated by the pairplot function from the *seaborn* package (v 0.12.1) in Python.

We used non-negative matrix factorization (NMF)[57] with custom k-means clustering (NMF*k*)[58] to identify signature components from the 7312 and 5515 SOM molecular formula (for surface and subsoil, respectively) we detected (i.e., *N* formulae in *m* soils) with *pyNMFk* package (Python, https://github.com/lanl/pyDNMFk, Figure 1). More details on NMF*k* assumptions, model settings, and model robustness are in the Supporting Information. Briefly, NMF*k* tends to be more successful at extracting explainable basis or signatures from large multivariate datasets, compared to other dimensionality reduction tools such as principal component analysis.[57, 59] As applied here, NMF*k* summarizes data into discrete signatures that contain weights for each SOM formulae detected by FTICR-MS for each soil layer independently (i.e., a separate set of signatures was generated to summarize surface versus subsoils, allowing us to explore depth-specific relationships with potential soil respiration). The optimal number of signatures was determined from silhouette coefficients of different NMF*k* models. A W-matrix with the weights of each SOM formulae (*N*) to each extracted signature (*k*), and an H-matrix with the contribution of each signature (*k*) to each soil sample (*m*) were generated from NMF*k*. To visualize the composition of each NMF*k* signatures (W-matrix), we generated a heatmap of SOM formula with normalized weights (0-1) >0.5 in at least one NMF*k*, clustered by van Krevelen class assignment (clustermap function from seaborn package). Within each inferred chemical class of SOM formula, we further clustered formula using the “linkage” method from the *scipy* package (“ward” method with “Euclidean” distance) to illustrate the difference between NMF*k* signatures.

To define groups of soils with high, medium, or low rates of potential respiration, we used *k*-means clustering on potential soil respiration with the elbow method to select the number of groups (*KMeans* from *scikit-learn* package).[60] Then, we mapped the extracted k signatures to soil respiration using supervised machine learning. To evaluate the potential value of NMF*k*-extracted SOM signatures for explaining soil respiration, we conducted three sets of machine learning models: (1) selected environmental parameters alone (i.e., variables with R^2 >0.2 in individual regressions, Figure 2, Table S1), (2) SOM composition alone (NMF*k* weights from H-matrix), and (3) environmental and SOM composition in combination. All machine learning models were built using gradient boosting regression (GBR) from *scikit-learn* package (v. 0.24, Python). More details in model training, testing and validation are in Supporting Information.

Results

Soil physicochemistry and potential respiration

Overall, many soil parameters, including potential soil respiration, tended to be higher in surface soils than in subsoils. Significant differences ($p<0.05$) between surface soils and subsoils in total C, total N, total sulfur, C/N ratio, and other factors are shown Figure S3. In particular, surface soils had higher potential respiration rates (median: 72.6 ug CO_2/g soil/day) than subsoils (median: 21.9 ug CO_2/g soil/day) (Mann–Whitney U = 3022.5, $N_{surface}$ = 63, $N_{subsoil}$ = 61, $P < 0.05$).

We grouped potential soil respiration into 3 levels corresponding to low, medium, and high respiration in each soil layer using k-means clustering (Figure S2). For both surface and subsoils, soil with high potential respiration tended to be sourced from the Midwestern and Northeastern United States. (Figure 3, Figure S5). In surface soil, high potential respiration was associated with five soils collected in Utah, Wyoming, and Virginia (within temperate conifer forest and temperate broadleaf & mixed forest biomes, Figure 3, Figure S1). In subsoils, high respiration was associated with three soils from Utah and Maryland (temperate conifer forests and broadleaf & mixed forests biomes). Desert soils had the lowest respiration in both layers (Figure S1).

We found relationships between soil respiration and many variables that supported prevailing paradigms. A full correlation table of associations between different soil properties is available in the SI (Table S1). Briefly, potential respiration rates in both surface and subsoils were positively correlated with gravimetric water content (GWC) (r^2: 0.246 and 0.225, $p<0.05$) and cation exchange capacity (CEC, r^2: 0.405 and 0.354, $p<0.05$, Figure 2). They were also positively correlated with total C and total N content, with stronger relationships in surface soils (r^2: 0.487 v.s. 0.268 for total C, r^2: 0.439 v.s. 0.248 for total N, $p<0.05$). Total bases and magnesium (Mg) concentrations had a higher correlation to respiration in subsoils than surface soils (r^2: 0.227 v.s. 0.146 and 0.287 v.s. 0.160, $p<0.05$, Figure 2), while manganese (Mn) concentrations were correlated to respiration in surface soils (r^2: 0.324, $p<0.05$, Figure 2).

SOM composition and NMFk partitioning of SOM.

Across all soils, the most common chemical classes of SOM were lignin-, condensed hydrocarbon-, and tannin-like formula. Most formula in these classes were present in both surface and subsoils (i.e., ‘shared’ formula). However, surface soils contained more unique formula than subsoils for all compound classes (Figure 3b). In particular, many protein-, amino sugar-, and lipid-like compounds were identified in surface soils only, with very few compounds in these classes being unique to subsoils. Because SOM consists of thousands of different compounds, we also used alpha diversity to represent the SOM richness per sample (Figure 3). Soils from the Midwestern U.S. and the West Coast had relatively higher alpha diversity than soils from other regions.

We used NMF*k* to summarize SOM composition into 7 and 5 NMF*k* signatures, respectively, for surface and subsoils (Figure 4). Geographic patterns in SOM signatures are displayed in Figure S6-7, with more geographic clustering of NMFs in surface soils than in subsoils. For surface soils, NMF3 presented as the largest relative contributor to SOM composition in 20 soils across all biomes (i.e., highest weighting in H-matrix, hereafter, ‘dominant signature’, Figure S6). NMF2, NMF5, and NMF7 served as the dominant signature in at least 9 soils each. For subsoils, NMF5 and NMF2 were the dominant signature in 27 soils and 16 soils respectively distributed across all biomes in the continental United States. There was no single NMF signature that could exclusively represent SOM composition of all sites in the same region for either surface or subsoils, suggesting that SOM composition at local sites is best summarized by a combination of multiple NMFs.

The most important formula contributing to the composition of each NMF (i.e., formula with normalized weights >0.5 in W-matrix) are shown in Figure 4a-b. For surface soils, NMF1, 4, 6, and 7 had a relatively higher number of important compounds identified as lignin-like. NMF6 and 7 had larger contributions of condensed hydrocarbon-like formula. NMF1 had higher contribution from protein-like and amino sugar-like compounds, while NMF3 and 5 had the lowest contribution from protein-like, amino sugar-like, and lipid-like compounds, suggesting their low microbial activities. NMF4 had the largest number of lipid-like compounds as important features. In subsoil samples, important formula for all NMFs tended to be classified as lignin-, tannin-, and/or condensed hydrocarbon-like. NMF1 and NMF5 had more features identified as lignin-like and some tannin-like compounds. NMF2 had the largest fraction of condensed hydrocarbon-like compounds. NMF4 had larger contributions of protein-like and amino sugar-like formula (Figure S8). We also compared if formula contributing to NMF signatures tended to be similar among surface and subsoils by assessing shared vs unique formula. NMF-selected formula (weights >0.5 in W-matrix) followed the same general patterns as the overall SOM pool but showed amplified relationships (Figure 4c).

We also observed differences in dominant NMF signatures across high-, medium-, and low-respiration soils, particularly in surface soils (Figure 4d-e). High respiration surface soils were characterized by five NMF signatures (1, 2, 3, 6, and 7), with the largest contribution from NMF6. Low respiration surface soils, in contrast, uniquely contained NMF5, and they did not have any contribution from NMF6. In subsoils, high respiration soils consisted of NMF 1, 2 and 4, while low respiration soils consisted of NMF1, 2, and 5. NMF5 had a larger contribution in low-respiration soils from both temperate forests and grasslands/shrublands. NMF5 had higher weights in low-respiration soils, and NMF3 and NMF4 had lower weights in low-respiration soils.

Relative importance of physicochemistry and SOM composition in potential soil respiration models

We developed gradient-boosting regression models to predict potential soil respiration with (1) physicochemical variables, (2) SOM composition represented by NMF signatures, and (3) both of them combined. Model performances are summarized in Table 1 and Figure 5.

Selected physicochemical variables (consisting of total C, total N, CEC, moisture, Mn (surface), total base (subsoil), and Mg (subsoil) concentration) had significant independent Pearson's correlation to respiration w/ $p < 0.05$ and $r^2 > 0.2$ (Table S1). Physicochemical variables predicted potential respiration rates in surface and subsoils equally well ($R^2 = 0.44$ and 0.43 respectively for testing data). In surface soils, total C, total N, and cation exchange capacity (CEC) were identified as the top 3 most important predictors, followed by Mn concentration and soil moisture (Figure 4). In subsoils, CEC, total N, and soil moisture were the most important predictor, and total C was the least important predictor (Figure S9).

Using SOM composition (NMF signatures) as predictors, we had better model performance in surface soils than in subsoils (testing $R^2 = 0.54$ vs. 0.08), and SOM composition alone predicted more slightly variation in potential respiration rates than physicochemical variables alone in surface soils (testing $R^2 = 0.54$ vs. 0.44), even when controlling for an equal number of predictors (testing $R^2 = 0.48$ vs. 0.44). NMF3, NMF5, and NMF2 were the most important SOM composition variables for explaining soil respiration in surface soils (Figure 5).

When we combined both physicochemical variables and SOM composition into a single predictor set, we obtained better respiration model performance ($R^2 = 0.62$) compared to models with environmental variables or SOM composition in surface soils only. However, the model describing potential respiration rates in subsoil was worse ($R^2 = 0.36$) when compared to models based on physicochemical variables only. In surface soils, the 3 most important variables were the same as the physicochemical model (Figure 5). NMF6 was identified as the most important SOM variable, followed by NMF3, NMF2, and NMF5 (Figure 5).

Discussion

Depth partitioning in relationships between SOM composition and potential soil respiration

Given that not all chemical constituents of SOM contribute to soil respiration and that surface and subsoils differ substantially in mineralogy and structure, we hypothesized that distinct subsets of SOM would contribute to respiration in surface vs. subsoils. There was no single NMF that dominated low- vs. high-potential respiration soils in either layer, however, NMF weightings varied substantially across soils with different rates of potential respiration in both layers (Figure 4d-e). This suggests that different subsets of SOM were disproportionately associated with soils exhibiting high vs. low potential respiration rates. While patterns in SOM chemical across geographic regions were difficult to disentangle, the spatial distribution of NMF types suggested local similarity in SOM composition in both layers (Figure S6-7), likely reflecting similar underlying chemistry, mineralogy, and/or biogeochemical processes.[61]

The SOM formula within NMFs that correspond to changes in soil respiration may represent a key step forward in understanding the chemical bioavailability of water-extractable organic matter in soils. In surface soils, NMF6 displayed a dramatic increase in weightings from low-to-high respiration soils. It contained a diverse suite of compounds including protein-, (soluble) lipid-, and amino sugar-like formula that can be rapidly used as microbial substrate. Proteins and amino sugars can fuel microbial metabolism of SOM,[62, 63] thus the prevalence of these compounds within NMF6 may support high potential rates of soil respiration. NMF1 and NMF7 in surface soils contained a diverse mixture of compounds and also increased from low-to-high respiration soils, supporting a possible relationship between SOM pool diversity and microbial respiration (see previous section). In contrast, surface NMF2, NMF3 and NMF5 decreased in importance from low-to-high respiration soils and primarily consisted of a small but unique subset of lignin- and tannin-like compounds (Figure 4a). This is consistent with low bioavailability of its chemical constituents suppressing microbial respiration.[64, 65] It suggests that despite the often-inferred high bioavailability of water-extractable SOM,[41, 66] there may be a significant fraction of water-extractable SOM that is chemically protected from microbial decomposition.[40, 41, 63] Interestingly, NMF4 in surface soils — which contained the greatest number of lipid-like formula (Figure 4a) and had a comparatively large fraction of protein-like formula —was not present in any high-respiration soils. We therefore suggest that NMF4 may be an indicator of non-living microbial biomass (i.e., necromass) which is disproportionately comprised of lipids (microbial cell wall remnants) and amino sugars and proteins (the basis of intracellular materials).[67, 68] The comparatively weak relationship between subsoil water-extractable SOM and potential soil respiration as compared to surface soils highlights recent work emphasizing the importance of mineral-associated organic matter in soil C storage.[69-71]

Relative importance of physicochemistry and SOM composition in predicting potential soil respiration

By developing machine learning models to predict respiration with soil physicochemistry and SOM composition (NMFs) separately and in combination, we were able to distinguish the contributions of each set of factors for predicting soil potential respiration. The models based on physicochemistry alone explained a modest amount of variation in soil respiration (44% and 43% in surface and subsoils, respectively), in line with the range of explanatory power observed in other works.[72, 73] For surface soils, models based on SOM composition alone (54% variation explained) and both physiocochemical factors and SOM composition combined (62% variation explained) suggest that SOM composition (1) can predict soil respiration at least as well as commonly measured physiocochemical variables and (2) explains some portion of soil respiration that is not captured by physiocochemistry. In models based on SOM composition alone, NMF3 (which was mainly in low-respiration soil and was comprised of lignin- and tannin-like formula, see previous sections) was the strongest predictor of soil respiration followed by NMF2 and NMF5. The relative chemical recalcitrance of the most important predictors of respiration may suggest that the proportion of thermodynamically unfavorable formula in water-extractable SOM has a direct inhibitory effect on soil metabolism. Indeed, thermodynamic regulation of organic C composition can be a key control for the rate of respiration in ecosystems.[40, 41] Therefore, the inclusion of SOM composition in more mechanistic modeling approaches may be able to improve predictions of soil respiration rates.

However, models for subsoils displayed different dynamics. In the subsoil model based on physicochemical variables alone, total C was the least important predictor (vs. the most important predictor for surface soils), and the model containing SOM composition did not yield high predictive power. The marginal effect in partial dependence of surface soil respiration to total C was stronger than the effect of subsoil respiration (Figure S10), supporting a stronger association between total C and potential respiration in surface soil vs. subsoil. The low predictive power of total C relative to other physicochemical factors could explain why SOM composition did not add predictive power to potential respiration in subsoils. Since more total and organic C is stored in surface soils, resolution into the water-extractable SOM pool (reflected here by NMFs) might be a more significant factor for predicting surface soil respiration than in subsoils that are characterized by lower total C and more mineral-associated SOM.[74]

Our results suggest that NMF-extracted signatures of SOM composition are able to improve surface soil model performance by integrating fundamental molecular information into soil respiration models across very different soil ecosystems at the continental scale. NMF6, which was the most important NMF signature in combined models of surface respiration, consisted of diverse chemically-bioavailable compounds, and it mainly existed in high-respiration soils (see previous sections).[65] We therefore suggest that chemically-bioavailable compounds in water-extractable SOM pools may provide the greatest complementary explanatory power to physicochemical factors in respiration predictions. Because SOM pools vary tremendously at the continental-scale, refined regional or local studies that encompass lower-variability parameter spaces may yield even more value of SOM molecular data to soil C modeling.

We note that physicochemical predictors were stronger predictors of soil respiration than SOM composition in the combined surface soil models. However, the inclusion of SOM composition improved physicochemistry-only models by 18%, indicating that it may significantly impact our ability to predict the rate of soil C cycling processes. Future modeling with carefully applied machine learning approaches may open up new avenues for further extracting the relevant portions of SOM pools for inclusion in climate models.

Conclusion

Leveraging molecular information of SOM chemistry to improve conceptualizations and models of soil C cycling is a pressing challenge for global biogeochemical and climate predictions. In this study, we use machine learning (NMF*k*) to distill the thousands of SOM molecules detected by ultrahigh resolution mass spectrometry in soil cores across the continental United States into tractable units. These signatures of SOM composition represent different subsets of SOM composition that contribute differently to soils exhibiting low versus high rates of potential respiration. We then disentangle the SOM formula from each NMF*k*-extracted signatures and validate their chemical recalcitrance in microbial respiration in surface soils. Additionally, SOM chemistry (as summarized by NMF*k*) explained a greater proportion of potential soil respiration than commonly measured physicochemical factors, and provided additional explanatory power beyond these factors in combined models. Our results provide a basis for molecular information to spur the development of new process-based modeling of soil C cycles and underscore the role of specific chemical constituents within the water-extractable SOM as a determinant of soil respiration.

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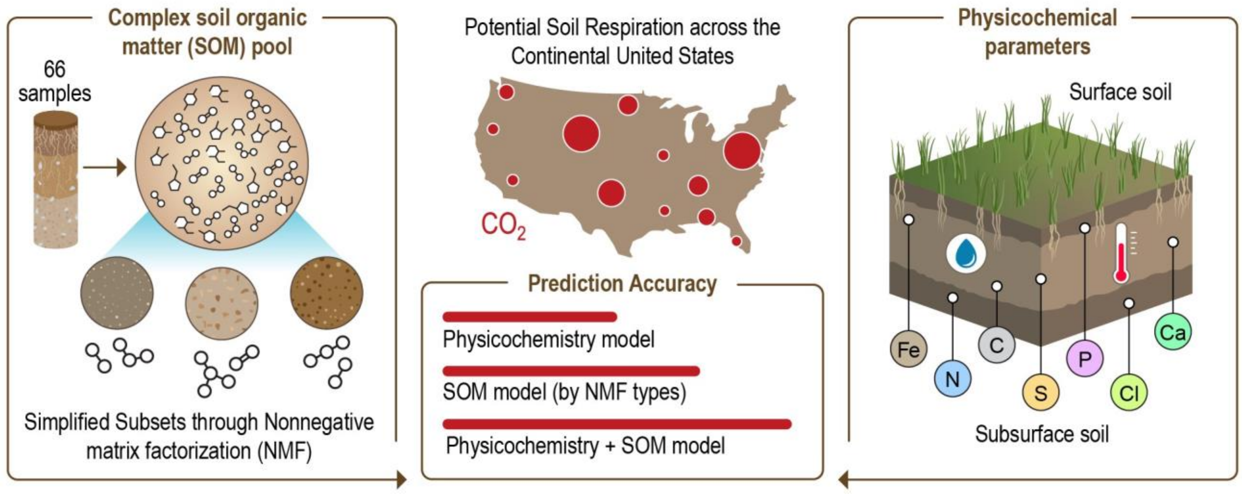


Figure 1. Proposed workflow: Machine learning models summarize molecular data to predict soil respiration. Non-negative matrix factorization (NMF*k*) extracts key SOM signatures from high resolution mass spectrometry measurements of SOM. Gradient boosting regression predicts soil respiration with physicochemistry, SOM signatures, and physicochemistry combined with SOM signatures.

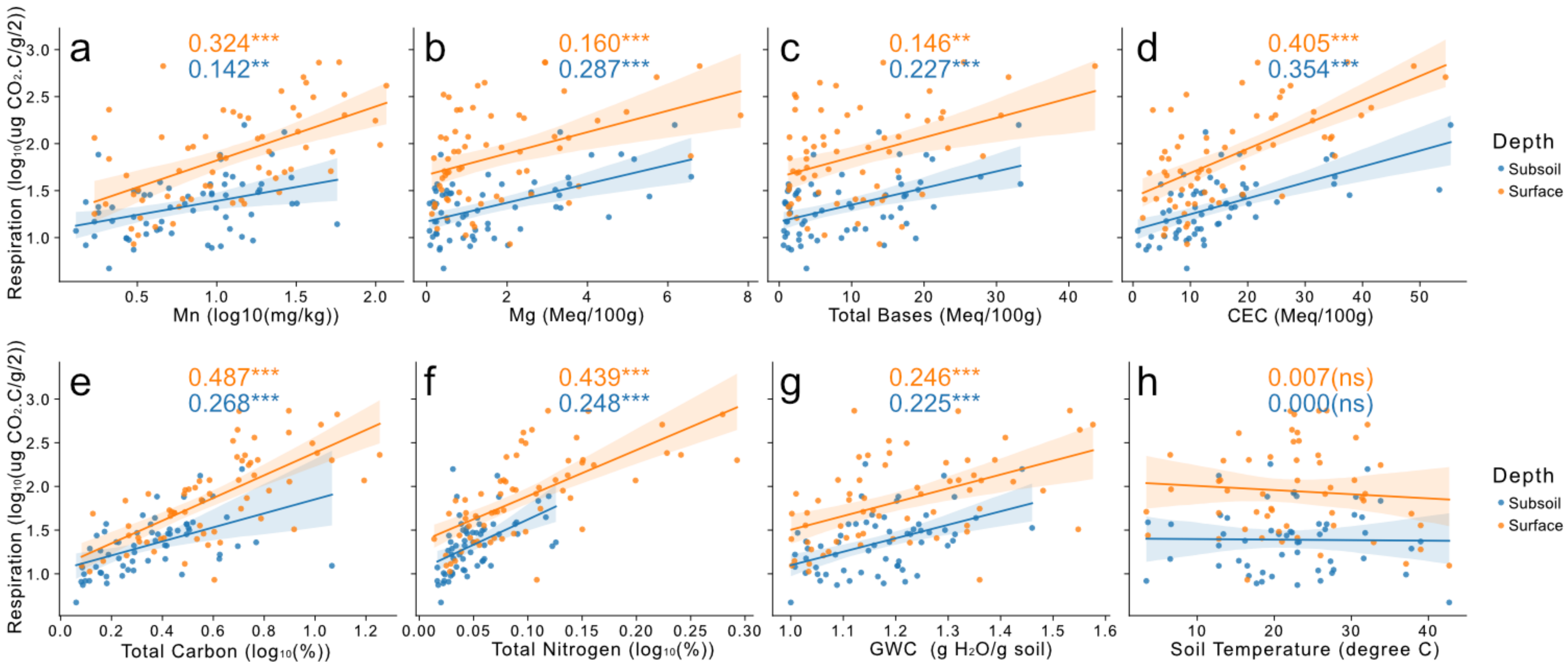
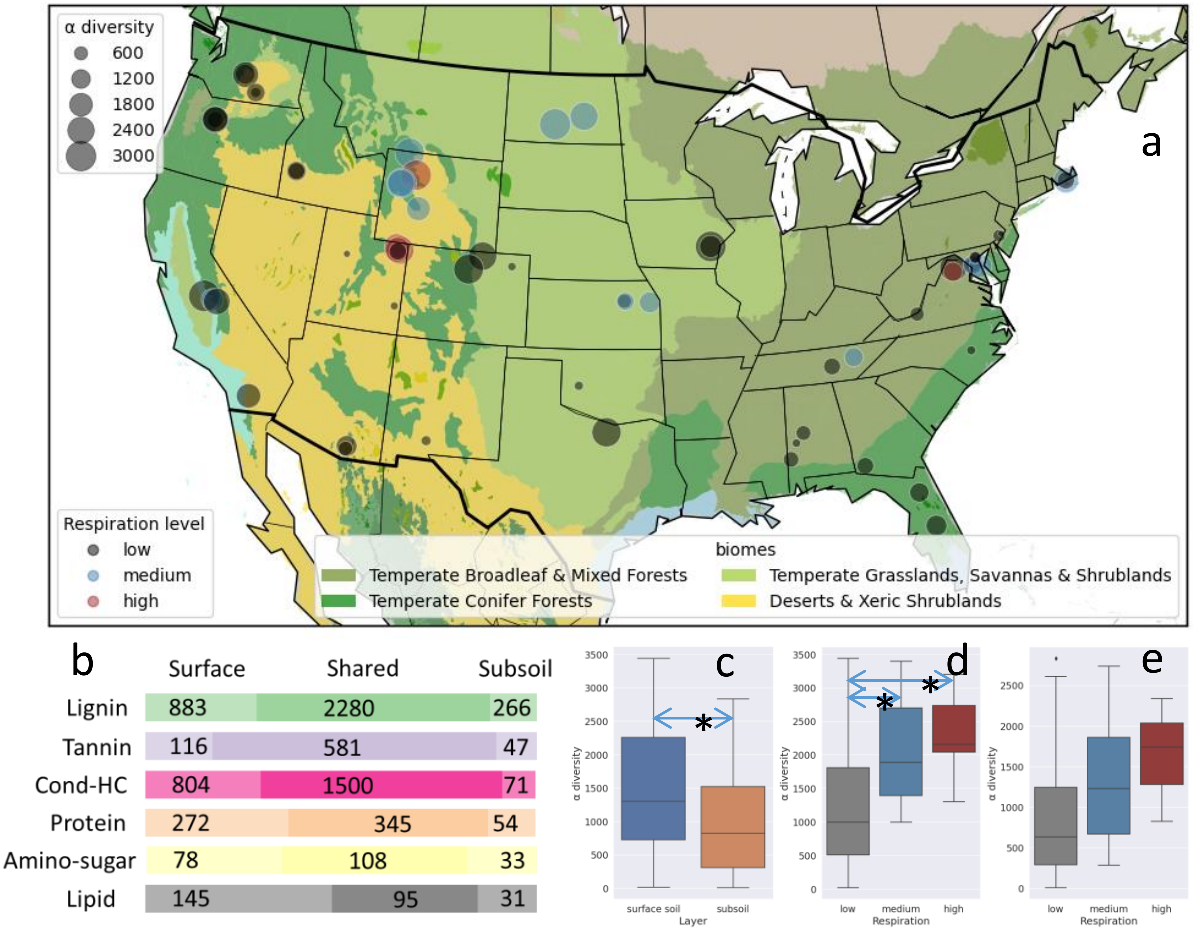


Figure 2. The relationship between soil characteristics and potential respiration. (a-h) show [Manganese(Mn), Magnesium(Mg), Total Bases, CEC, Total C, Total N, GWC, Soil Temperature], respectively. Orange represents surface soils and blue represents subsoils. Lines denote the fitted linear regression function. Numbers on each panel are r^2 value from linear regression, the stars behind represents statistical significance (*** ($p \leq 0.001$), **($p \leq 0.01$), ns ($p > 0.05$)).



	Surface	Shared	Subsoil
Lignin	883	2280	266
Tannin	116	581	47
Cond-HC	804	1500	71
Protein	272	345	54
Amino-sugar	78	108	33
Lipid	145	95	31

Figure 3. (a) Spatial distribution of soil respiration levels (labeled by colors) and alpha diversity of each sample (sizes). Soil respiration levels are determined by *k*-means clustering on soil respiration rates (ug CO2/g soil/day). Soils from temperate conifer forests and temperate grasslands, savannas & shrublands have relatively higher respiration rates compared to other biomes (Figure S1). (b) The number of shared and unique SOM compound classes identified between surface and subsoils. The classes were suggested by van-Krevelen plot. (c) The difference of alpha diversity in surface and subsoil soils ($p < 0.05$ from ANOVA, *: $p<0.05$ from Tukey's HSD test) (d) the difference of alpha diversity in surface soils with different levels of potential respiration ($p < 0.05$ from ANOVA, *: $p<0.05$ from Tukey's HSD test) (e) the difference of alpha diversity in subsoils with different levels of potential respiration ($p < 0.05$ from ANOVA).

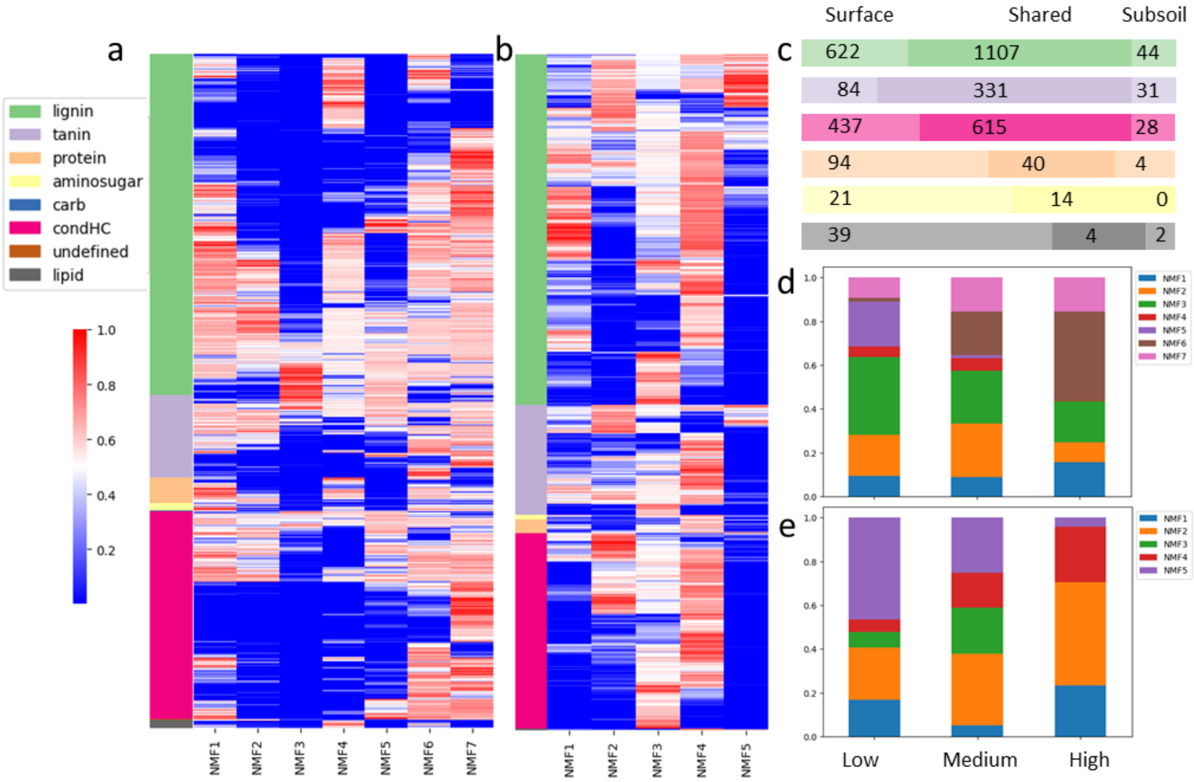


Figure 4. NMF*k* partitioning of SOM composition. (a-b) Relative contribution of organic formula to each SOM signatures identified by NMF*k* in a) surface and b) subsoils. The color in each cell represents the normalized (0 to 1) relative contribution for each SOM feature (row) to each NMF*k* signature (column), red indicates the most important contributor, and blue indicates the least. The side bar indicates the compound class of each SOM feature. (c) The number of shared and unique formula identified as important (normalized weights >0.5) by NMF*k* in surface and subsoils. (d-e) The relative contribution of NMF*k* signatures to each level of respiration rates in both d) surface and e) subsoils. Surface soils: low respiration level (N = 44), medium respiration level (N = 14), high respiration level (N = 5, UT12, UT23, UT24, WY03, Temperate Conifer Forests, SCBI Temperate Broadleaf & Mixed Forests). Subsoils: low respiration level (N = 48), medium respiration level (N =10), high respiration level (N = 3, T12, UT19, Temperate Conifer Forests, WLLO, Temperate Broadleaf & Mixed Forests).

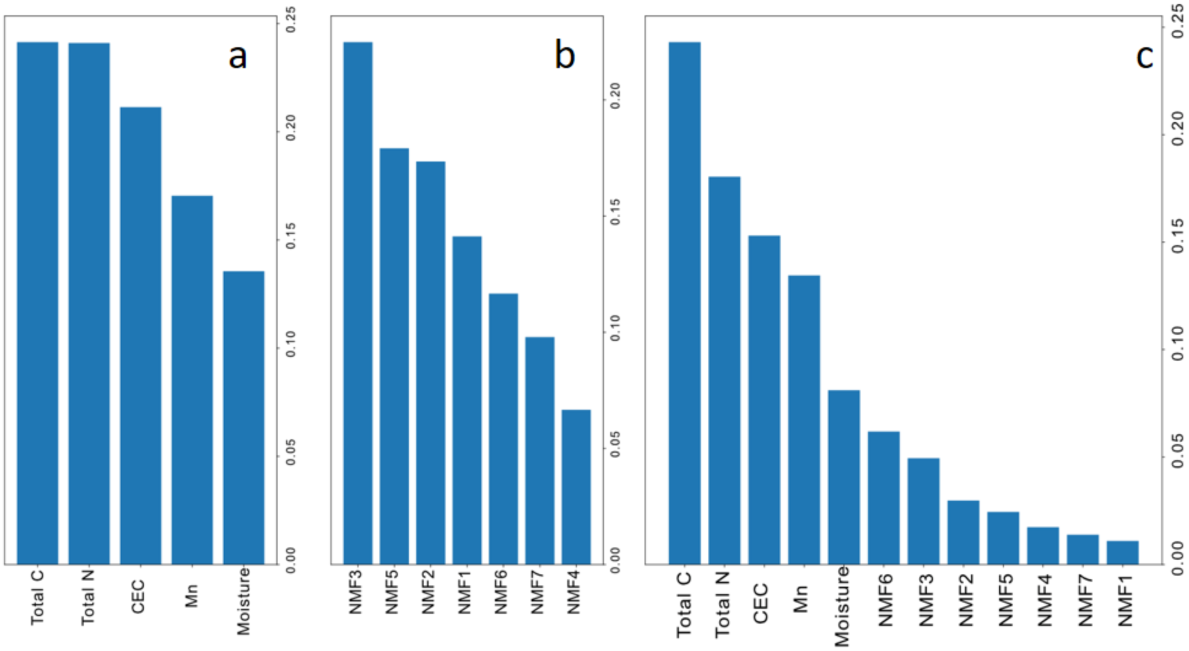


Figure 5. Relative importance of each predictor in surface soil potential respiration machine learning models. a) Physicochemistry model, with physicochemical variables only. b) SOM model, with SOM signatures represented by NMFs only. c) Physicochemistry & SOM model with both physicochemical variables and SOM signatures.

Table 1. Model performance for predictions of potential soil respiration with physicochemical variables (Physiochemistry model), SOM by NMF*k* signatures (SOM_model), and combined physicochemical variables and SOM variables (Physiochemistry &SOM_model) for average 5-fold cross-validation accuracies (training soils, RMSE), and testing sample accuracies (RMSE, R2).

	Physiochemistry Model	SOM_model	Physicochemistry &SOM_model
Surface_CV	0.80	1.05	0.82
Surface_test	0.98	0.89	0.82
Surface_test (R2)	0.44	0.54	0.62
Subsoil_CV	0.60	0.82	0.67
Subsoil_test	0.46	0.80	0.49
Subsoil_test (R2)	0.43	0.08	0.36