**Poorly crystalline mineral content is more important than parent material or climate alone for explaining soil C dynamics**

**Key messages:**

* Climate effect explains more variance in surface ∆14C, but parent material explains more at depth
* Parent material and climate interact to control both bulk 14C and respired 14C-CO2
* Poorly crystalline mineral content is highly correlated with the difference between bulk soil 14C and respired 14C-CO2

**Introduction**

* Climate change effects on soil C will determine whether or not soils continue to be a sink or will become a source in the coming decades
  + Understanding these effects requires understanding soil carbon persistence mechanisms
  + This study specifically looking at parent material, climate, and their interaction via weathering
* Climate most important explanatory factor for global variation in C stocks and 14C
  + Function of temperature and moisture effect on decomposition rates
* Mineral association attenuates these effects
  + Examples of decomposition rates different for different parent materials under the same climate conditions
  + Lab and field?
  + E.g....
* Mechanisms of mineral protection and specific minerals, i.e. beyond clay
  + Poorly crystalline minerals in particular
  + Develop from interaction of parent material and climate over time, i.e. weathering
  + Examples of mechanisms
* Bulk soil 14C as proxy for age
  + Larger pools that cycle more slowly dominate mass of bulk soil C, but contribute less to respired C fluxes
  + 14C of heterotrophically respired CO2 gives relative contribution of faster or more slowly cycling soil C to flux
  + Respired 14C emphasizes contributions from smaller, fast cycling pools
  + Therefore difference between two provides a measure of the degree of protection of soil C (or presence of C pools that are persistent over centuries-millennnia because of strong mineral protection)
* Introduce sites
  + ...
* Expectations for effects of parent material and climate effects
  + Linear models (?)
  + Teaser of findings, importance of weathering for mineral assemblage;
  + Interaction of parent material and climate key for understanding the variance in 14C of both bulk and respired C
  + Show how poorly crystalline minerals implicated in soil C protection

**Methods**

*Site descriptions*

We collected samples from 9 sites across a combined gradient of parent material and climate in the Sierra Nevada Mountains of California (Table 1). These mountains provide natural independent gradients of parent material and mean annual temperature (MAT). Moving from north to south along the cordillera the parent material changes from basalt to andesite to granite, while the change in elevation headed eastward from the Central Valley leads to a decrease in MAT that is consistent for each parent material.

Total mean annual precipitation is relatively constant with elevation, but falls mainly as rain at lower elevations, and mainly as snow at higher elevations. There is a slight precipitation gradient running north to south, with MAP of xxx mm yr-1 averaged across the basalt sites in the north, xxx mm yr-1 for the andesitic sites, and xxx mm yr-1 for the granodiorite sites in the south.

Vegetation at the study sites is typical of the Sierra Mixed Conifer habitat (*Parker, I., and W. J. Matyas. 1981. CALVEG: a classification of Californian vegetation. U.S. Dep. Agric., For. Serv., Reg. Ecol. Group, San Francisco.)*. All of the sites are forested and dominated by conifers, although the species composition changes along with climate. Tree species at the lowest elevation, “warm”, sites are predominantly *Pinus ponderosa* mixed with lesser amounts of *Quercus* spp. The canopy species at the mid-elevation cool sites is comprised primarily of *Abies concolor* and *Pinus lambertiana*, while *Abies magnifica* is the dominant species at the highest elevation “cold” sites. Species present at all sites include *Calocedrus decurrens* in the canopy, the shrubs *Arctostaphylos* spp., *Chamaebatia foliolosa*, and *Ceanothus* spp. to varying degrees, and ground cover of grasses and forbs.

*Sample collection*

Site locations were initially established in 2001 by C. Rasmussen (Rasmussen et al., 2006), and resampled in 2009 (Rasmussen et al., 2018). We returned in late September of 2019 to collect samples for this study. Sites were located using GPS and geospatial coordinates recorded during site establishment. At each site we dug three replicate pits down to a depth of 0.3m. Prior to sample collection we compared the soil profiles to the pedon descriptions from the previous sampling campaigns. After confirming profiles were comparable we collected samples from the pit sidewalls in 0.1m increments for each of the three pits. We also measured the depth of the litter layer and collected representative litter samples from each site.

*Spline fitting*

Soils collected in 2001 and 2009 were sampled by horizon, while soils collected in 2019 were sampled by depth. We were motivated to use consistent depth increments across sites because of the strong correlation between depth and 14C. In order to make the horizon and depth-based measurements comparable, we fit a mass-preserving quadratic spline to the 2001 and 2009 profiles in order to convert soil property data to the equivalent depth increments sampled in 2019 (Bishop et al., 2001). We used the mpspline function of the GSIF package in R, with a lamba value of 0.1 (Hengl 2019).

*Incubations*

Laboratory soil incubations were performed on composite samples from the three replicate pedons sampled at each site. We composited and incubated each depth increment (0-10cm, 10-20cm, 20-30cm) separately in 1 L glass mason jars fitted with sampling ports in the lids. Incubations were performed in duplicate. Prior to the start of incubations we adjusted the soil moisture content to 60% of water holding capacity (WHC). We defined WHC as the gravimetric water content of water-saturated soil placed in mesh-covered (50µm) tubes (50ml) weighed after draining for 30 minutes on a bed of fine sand. Following rewetting we allowed the soils to respire for one week before closing the jars. Incubations proceeded until CO2 concentrations in the jar headspace reached approximately 10,000 ppm, at which point we collected a 400ml gas subsample for radicocarbon analysis. Gas samples were collected with pre-evacuated stainless-steel (Restec) vacuum canisters. All incubations were performed in the dark at 20°C.

*Soil Physical Analyses and Mineral Characterization*

Data on soil particle size distributions, bulk density, and mineral characterization were obtained from previously published analyses of samples collected at the study sites in 2001 and 2009 (Rasmussen et al. 2006, Rasmussen et al., 2018). Both qualitative and quantitative approaches were used to characterize soil mineral assemblages, including X-ray diffraction (XRD) for the clay (<2µm) fraction, atomic absorption spectroscopy, and non-sequential selective dissolution. Details on these analyses are provided by Rasmussen et al. (20XX). In this study we use the amount of iron selectively dissolved from bulk soils by acid ammonium-oxalate and citrate dithionite as proxies for the quantity of poorly crystalline and crystalline minerals, respectively.

*Carbon, Nitrogen, and Radiocarbon Analysis*

Total carbon and nitrogen content was determined by dry combustion (Vario Max, Elementar Analysensysteme GmbH) on finely ground soils (Retch MM400). For radiocarbon analysis, we first purified CO2 from incubation flask samples and combusted soil samples on a vacuum line using liquid N2. Following purification, samples were graphitized with an iron catalyst under an H2 enriched atmosphere at 550°C. Radiocarbon content was then measured by accelerator mass spectrometry (Micadas, Ionplus, Switzerland) at the Max Planck Institute for Biogeochemisty (Steinhof, 2017).

We report radiocarbon values using units of ∆14C, defined as the deviation in parts per thousand of the ratio of 14C–12C from that of the oxalic acid standard measured in 1950. This unit also contains a correction for the potential effect of mass-dependent fractionation by normalizing sample δ13C to a common δ13C value of 25 per mil (Stuiver & Polach, 1977). Values with ∆14C >0 indicate the presence of ‘bomb’ C produced by atmospheric weapons testing in the early 1960s. Values with ∆14C < 0 indicate the influence of radioactive decay of 14C, which has a half-life 5730 years.

*Statistical analysis*

We used a linear modeling approach to assess the relative explanatory power of climate versus parent material on the observed variation in 14C, as well as potential interactions between these two factors. We constructed separate models for bulk soil 14C and respired 14C-CO2. For each model we considered the two-way interaction between parent material and climate as well as the three-way interaction with time. For ease of interpretation, we considered the effect of depth by modeling each depth layer separately (0-10 cm, 10-20 cm, 20-30 cm). We also made pairwise comparisons of bulk 14C and respired 14C-CO2 across sites and within years, as well as comparisons of individual sites across years. We assessed the significance of the temporal trend for pairwise combinations of parent material and climate using the emmtrends function of the emmeans package (Lenth, 2021). We corrected for multiple comparisons using Tukey’s honestly significant mean difference.

We assessed the relative importance of poorly crystalline versus crystalline iron minerals in protecting soil C from microbial decomposition by regressing the difference between bulk soil 14C and respired 14C-CO2 against the concentrations of ammonium-oxalate extractable iron, ammonium-oxalate extractable aluminum, pyrophosphate extractable aluminum, and dithionite-citrate extractable iron. We did not observe a significant relationship between the bulk and respired 14C difference and time, so we used 14C data from both 2001 and 2019 in the model. Selective dissolution was performed on splits of the soils collected in 2001. Extracted mineral concentrations did not change substantially by horizon, so in order to simplify data interpretation we conducted regressions for the entire 0-30 cm depth. We computed carbon mass-weighted means for bulk soil 14C data and flux-weighted means for the respired 14C-CO2 data.

**Results**

*Bulk soil radiocarbon*

Contrary to what would be expected from the decomposition-temperature relationship, bulk ∆14C of soils at the cool climate sites was equally or more depleted than what we observed at the cold climate sites (**Fig. 1** *depth profiles*). However, soils at the warmest sites tended to be the most enriched in ∆14C (**Fig. 1 a, d**). Comparing parent materials within climate zones, andesitic soils tended to be the most depleted in 14C, while the granitic soils tended to be the most enriched (**Fig. 1**).

The relationship of bulk soil ∆14C to atmospheric ∆14C appeared to be a function of both parent material and climate. In 2001, the warm climate sites were the only sites where the basaltic and andesitic soils were enriched relative to the atmosphere, and only for the uppermost soil layer (**Fig. 1a**). In contrast, the uppermost soil layers for both the warm and cool granitic sites were enriched relative to the atmosphere in 2001 (**Fig. 1a, b)**. Bulk ∆14C of all three lithologies were depleted relative to atmospheric ∆14C for both surface and subsoil layers at the cold climate sites (**Fig. 1c**). For the 2019 samples, surface soil ∆14C (0-10 cm) was enriched relative to the atmosphere at all sites except for the cool climate andesite soils (**Fig. 1, d-f**). At two of the sites in 2019, bulk soil 14C remained enriched relative to the atmosphere down to 30 cm: the warm climate granite soil (**Fig. 1d**) and cold climate basalt soil (**Fig. 1, f**). Bulk soil ∆14C at the cool andesite site was the most depleted relative to the atmosphere at all time points (**Fig. 1b, e**).

Linear models for bulk soil ∆14C revealed significant two-way interactions between parent material and climate at all depths (**Table 2** *anova*). Accordingly, we observed the greatest differences in bulk ∆14C among parent materials at the warm and cool sites (**Fig. 1a, b, d, e**). In contrast, bulk ∆14C at the coldest sites was the most similar across the three parent materials (**Fig. 1e, f**). Although ∆14C declined with depth for all sites, climate explained more of the variance for the uppermost soil layer (0-10cm) whereas parent material explained more in the bottom two layers (10-20cm, 20-30cm) (**Table 2** *anova*).

We also observed a significant three-way interaction between parent material, climate, and time at all three depths in the linear models for bulk ∆14C (**Table 2** *anova*). The change over time in bulk soil ∆14C was greater in magnitude at the surface than in the deeper soil layers (**Fig. 2** *timeseries*). For the uppermost soil layer we observed significant changes in both warm and cool climate granitic soils, as well as in the warm climate andesitic soils (**Fig. 2a**). In the deeper soil layers (10-20 cm and 20-30 cm) the only significant change over time was observed in the cool climate basalt and granite soils (**Fig. 2b, c**). Bulk soil ∆14C of the cool climate andesitic soils remained essentially unchanged between 2001 and 2019 for all depths (**Fig. 2a, b, c**).

*Heterotrophically respired radiocarbon*

The patterns we observed for respired ∆14C-CO2 were similar to what we observed in bulk soil ∆14C (**Fig. 1**). We found climate to be the most significant factor for explaining the variance observed in ∆14C-CO2 for the uppermost soil layer, as with bulk soil ∆14C-CO2, but unlike bulk soil ∆14C, parent material was not significant for explaining the variance in ∆14C-CO2 at this depth (**Table 2**). However, at the deepest depth, we found parent material to be more important than climate for explaining in ∆14C-CO2 variance (**Table 2**). Overall, the interaction between parent material and climate explained more of the variance in ∆14C-CO2 data than in the bulk ∆14C data (**Table 2** *anova*).

For the andesitic soils there were no significant differences in respired ∆14C-CO2 among climate zones at any depth (**SI Table X** *Tukey results for mm?***)**. In contrast, ∆14C-CO2 respired by the basaltic and granitic soils diverged substantially between climate zones, particularly for the 10-20 cm and 20-30 cm depth layers (**Fig. 1**). Overall, 14C-CO2 across sites was most similar at the soil surface, and most divergent at the intermediate depth (10-20 cm) (**Fig. 1**).

We observed the greatest changes in ∆14C-CO2 over time at the soil surface (**Fig. 2, d**), and these changes were significant for the majority of sites. In absolute terms, the changes in ∆14C-CO2 over time in these uppermost soil layers were greater at the warm sites (-3.6 per mil ±2.1 yr-1) than at the cool sites (-2.3 per mil ± 2.4 yr-1), and greater at the cool sites than the cold sites (-0.7 per mil ± 1.6 per mil yr-1). Overall, the changes in ∆14C-CO2 that we observed in this study were typically lower in magnitude than the change in atmospheric ∆14C (-5.2 per mil yr-1) over the same time period.

The soils for which we observed the most depleted ∆14C-CO2 were from the cold climate granite site (**Fig. 1c, f**). These sites were also the only ones to show clear enrichment in respired ∆14C-CO2 from 2001 to 2019 (**Fig. 2e, f**). We measured highly depleted ∆14C-CO2 in the deeper soil layers in both 2001 and 2019: -469.1 and -127.8 per mil for the 8-27 cm layer in 2001, and -396.7 and -23.5 per mil for the 10-20 cm layer in 2019 (**SI Table X**). We do not believe this was due to laboratory error, given that it was consistent over time and restricted to the deeper soil layers from this one site. However, since it appears to be a unique response to these soils, we have excluded these highly depleted samples from the statistical analyses.

*Bulk and respired 14C differences*

Respired 14C-CO2 was enriched relative to bulk soil 14C for all sites and at all depths with the exception of the soils at the cold granite site (**Fig. 1** *depth profiles*). The difference between bulk and respired 14C did not change substantially with depth for the granitic or basaltic soils, although it was slightly greater at depth for the andesitic soils. We did not observe substantial change in the differences between bulk and respired 14C over time either, when considered for the whole measured profile (0-30 cm) (**Table ?**). The one exception to this was for the cold climate basaltic soil, where the difference between bulk soil ∆14C and respired ∆14C-CO2 was 97.9 ± 13.3 per mil in 2001 and 26.2 ± 6.2 per mil in 2019.

Parent material had a greater impact than climate on the relationship of bulk soil ∆14C and respired ∆14C-CO2 in the regression analysis (ref equations?). For the parent material only model, we observed significantly greater enrichment of respired ∆14C-CO2 relative to the bulk soil ∆14C in the andesitic soils than in either the basaltic or granitic soils: *intercepts + CIs?* (**Fig. 3a** *bulk-inc regression*; SI table?). In contrast, we did not observe significant differences between climate zones in the climate only model **(Fig. 3b)**. However, the absolute differences between bulk and respired 14C tended to be smallest at the warm sites and greatest at the cool sites.

We also assessed whether interaction effects of parent material or climate with bulk ∆14C led to deviations from the 1:1 relationship with ∆14C-CO2. We found that changes in bulk soil ∆14C led to correspondingly smaller changes in ∆14C-CO2 for andesitic soils in the parent material only model (slope = 0.51, 95% CI = [0.22, 0.80]) (**Fig. 3a**), and for cool climate soils in the climate only model (slope = 0.61, 95% CI = [0.31, 0.90]) (**Fig. 3b**). While we could not directly test the interaction of parent material and climate factors in these models owing to the limited number of observations, mean differences in bulk ∆14C and respired ∆14C-CO2 were greatest for the soils with the combination of andesitic parent material and the cool climate zone by a large margin (Table? Numbers?).

*Mineral assemblages*

Mineral assemblage data is reported fully in Rasmussen et al. (2018). Here we focus on the selective dissolution data with respect to the trends we observed in bulk soil ∆14C, respired ∆14C-CO2, and the difference between bulk soil and respired ∆14C. We observed a significant negative correlation between the concentration of oxalate extractable iron and bulk soil ∆14C. Similarly, we also saw a significant negative relationship between bulk ∆14C and both oxalate extractable aluminum and pyrophosphate extractable aluminum (SI). For simplicity, we focus here on the oxalate extractable iron alone as a proxy for the abundance of poorly crystalline minerals. We used the difference of dithionite-citrate extractable iron and ammonium-oxalate extractable iron as a proxy for crystalline iron. In contrast to the poorly crystalline minerals, we did not find a significant relationship between the concentration of extractable iron from crystalline minerals and bulk soil ∆14C.

We did not observe any significant relationship between respired ∆14C-CO2 and any of the extracted metals. However, concentrations of oxalate extractable iron, oxalate extractable aluminum, and pyrophosphate extractable aluminum explained more of the variance in the difference between bulk soil and respired ∆14C than they did for bulk soil ∆14C alone. As with bulk soil ∆14C, there was no relationship between crystalline mineral abundance and the difference between bulk soil and respired ∆14C.

**Discussion**

In this study we considered the relative effects of parent material and climate on the distribution of radiocarbon in bulk soils and heterotrophically respired CO2 in order to determine how these factors control the cycling of soil C across timescales of years to centuries. Climate, and in particular temperature, has been found to be the most important variable aside from depth for explaining the distribution of ages of soil carbon at a global scale. Yet our current understanding of soil organic matter decomposition underscores the importance of physical mechanisms that may attenuate the classical temperature-decomposition relationship. Among the most salient of these mechanisms is the association of soil organic matter with minerals.

Soil mineral assemblages are the result of the weathering of primary minerals to form secondary minerals, and the preferential loss or stabilization of those secondary minerals over time. Differences in climate can therefore lead to different soil mineral assemblages when starting from the same parent materials, or to similar mineral assemblages despite starting from disparate parent materials. The relevance of soil minerals for mediating soil organic matter protection appears to be a function of the specific minerals present, rather than the amount of clay or total mineral surface area (Beyond Clay, etc.).

The soils we studied comprise a parent material by climate gradient, but also a weathering gradient in tandem with climate. The high elevation, cold climate soils in this study are poorly developed, while the cool climate soils are in intermediate stages of weathering, and the warm climate soils are highly weathered. Previous research characterizing the mineral assemblages at these sites using XRD and selective dissolution found that the dominant mineral species in the soils of the most weathered “warm” climate zone are 1:1 clays with large accumulations of crystalline iron oxides.

Parent material differences are most evident in the cool climate sites, with high contents of allophane in the andesitic soils, minor amounts of both 2:1 and 1:1 clays in the basaltic soils, and hydroxy-interlayered vermiculite in the granitic soils. The basaltic and andesitic soils found in the cold climate sites both had substantial amounts of SRO minerals, although with a greater concentration of 1:1 clay in the basaltic soils. In contrast, the cold climate soils developed on granite showed a mixture of HIV and kaolinite, but also a substantial accumulation of gibbsite.

In this study we considered the relative effects of parent material and climate on the distribution of radiocarbon in bulk soils and heterotrophically respired CO2 in order to determine how these factors control the cycling of soil C across timescales of years to centuries. Our key finding is that the patterns we observed in both d14C\_bulk and d14C\_resp could only be explained by considering the interaction of parent material and climate in addition to the effects of each factor alone. Furthermore, we found that we could explain this interaction quantitatively through the abundance of poorly crystalline minerals, which form and are preferentially retained at sites with soils in intermediate stages of weathering.

We found evidence for the overarching effects of climate on decomposition with respect to an increase in both soil C concentration and the apparent age of soil C (by proxy from d14c\_bulk) with decreasing temperature. However, the increase in soil C concentration with temperature was not linear, with substantially more C observed in the cool climate sites than in the warm climate sites, but similar soil C concentrations observed at the cool and cold climate sites. Furthermore, we observed the most depleted d14C\_bulk at the cool climate sites, rather than the cold climate sites. These results suggest that there is a mechanism at work in the cool climate zone soils that is slowing soil C decomposition, leading to correspondingly greater rates of soil C accumulation and increased soil C persistence than would otherwise be predicted on the basis of temperature alone.

We also observed differences in both soil C concentration and soil radiocarbon values due to parent material. The effect of parent material on soil C concentration was most pronounced at the cool and cold climate sites, while the effect on soil radiocarbon was most evident at the warm and cool climate sites. Overall, the andesitic soils were the most enriched in soil C and tended to have the most depleted values of d14C\_bulk. The granite soils tended to have the lowest soil C concentrations and d14C\_bulk values that were the most enriched, while the basalt soils were intermediate between the granite and andesitic soils with respect to both soil C concentration and d14C\_bulk. These findings highlight the importance of the initial mineral composition of a soil in determining both soil carbon accumulation and persistence.

Parent material is an important determinant of soil mineral assemblages, but due to weathering the mineral make-up of a soil changes over time. Rasmussen (2004) observed that mineral assemblages were the most similar among the three different parent materials in the highly weathered soils of the warm climate sites. These sites were also where we saw the least amount of differences in soil C concentration in the present study. In contrast, Rasmusssen (2004) observed the greatest differences in mineral assemblages at the cool climate sites, which is where we saw the strongest divergence from the expected relationship between temperature and soil C dynamics.

**Conclusion**

[change to focus on timescales rather than ∆14C trends?]

Our study shows clearly that parent material and climate interact to control soil C dynamics. This interaction was the key to explaining trends in both bulk soil ∆14C, which is a proxy for the mean age of soil C, and respired ∆14C-CO2, which reveals the relative contributions of faster or more slowly cycling soil C to respiration. We observed that the trends in both bulk and respired ∆14C at the soil surface were best explained by climate, but best explained by parent material in deeper soil layers. This importance of climate was reflected in the changes in ∆14C we observed over time, which were greater for surface soils than deeper soils, and greater for the highly weathered soils at the warm sites than the poorly developed soils at the cold sites. Yet contrary to expectations, we saw the most depleted bulk soil ∆14C in the cool climate andesitic soil, which is also where we saw the least change over time at all depths.

The interaction between parent material and climate can be characterized by the effects of weathering on the development of the soil mineral assemblage through formation and subsequent loss of secondary minerals. We confirm the findings of other studies that specific mineral assemblages are highly correlated with the age of soil C, as measured by proxy with bulk soil ∆14C. We extend this finding to show that it is specifically the poorly crystalline mineral content that is correlated with bulk soil ∆14C, while crystalline mineral content is not. Finally, we provide mechanistic evidence for the protective effect of mineral-association on decomposition of soil organic matter by demonstrating that the difference between bulk soil ∆14C and respired ∆14C-CO2 is even more strongly correlated with poorly crystalline mineral content than bulk ∆14C alone.

In future work we intend to quantify the timescales of soil carbon cycling in mineral and non-mineral associated pools with a compartmental modeling approach, using the radiocarbon time series presented here in addition to radiocarbon measurements of soil density and thermal fractions as constraints.