Roots as agents of soil change over time and as mediators of humanity's imprint on Earth's critical zone

By

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Abstract

Plant roots mediate the movement of materials such as nutrients and carbon (C) across Earth's surface. Roots allocate fixed C to processes that govern nutrient uptake, and in doing so redistribute soil resources. However, plant C for nutrient exchange strategies are not well understood and are proceeding amidst climate and land use changes that alter the amount of available C and nutrients, as well as the depth of root exploration. I examine the role of rooting system economies in nutrient availability and C cycling, and the ways those economies are changing as a consequence of anthropogenic activity. In Chapter 1, I characterize rooting systems' C for nutrient strategies across diverse forest ecosystems and demonstrate that, if left unperturbed, rooting systems increasingly tap into deeply distributed, mineral bound nutrient stocks over time to sustain forest nutrition. We even observed this effect in highly weathered soils where we would expect accumulating organic matter to play the primary role in ecosystem nutrition. Chapter 2 then characterizes the global extent of changes to rooting systems, demonstrating that human activity is inducing shallower rooting depths, on average, across the planet. Chapter 3 begins to address the biogeochemical consequences of shallower rooting systems in the Anthropocene by examining the C for nutrient economies in forests with and without extensive anthropogenic legacies at the Calhoun Critical Zone Observatory. There, I found that even after 80 years of forest regrowth, rooting systems developing after intense ecosystem disturbance remained truncated and thus reliant on shallow, organically-bound nutrient stocks, in contrast to unperturbed forests with deep roots that tapped into deep, mineral-bound nutrients. In Chapter 4, I examine the plasticity of rooting system C for nutrient strategies in the presence of distinct nutrient forms to better understand roots' capacity to cope with these changing soil environments. I found that rooting systems were able to adapt their C for nutrient

exchanges across varied depth distributions of mineral and organic nutrient pools, resulting in more C allocated at depths where the most potentially bioavailable nutrient forms reside. This suggests that plants' nutrient economies may be sufficiently plastic to respond to the novel nutrient depth distributions emerging with anthropogenic environmental change. Together, these studies demonstrate that rooting systems and their depth distributions are ecosystem traits that have unanticipated, outsized importance in governing the movement of C and nutrients within Earth's subsurface environments. In the context of the Anthropocene, these rooting depth-driven functions are subject to global perturbations that may promote wide-spread shifts in the ways nutrient and C cycles are structured from the ground up.

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General Introduction

Plants mediate the movement of nutrients across Earth's surface. In most ecosystems, many nutrients are found in minerals bound in rocks, from which they are released via weathering processes and subsequently transformed by biota into organic forms (Walker and Syers 1976, Crews et al. 1995, Vitousek et al. 1997). Plant roots are able to tap into both mineral- and organically-bound nutrients, rendering both forms of nutrients available for ecosystem productivity (Marschner and Rengel 2007, Lambers et al. 2008). Roots and their activities are thus important for liberating and distributing the nutrients necessary for all life.

Root-driven nutrient transformations depend in part on depth-dependent resource exchanges that plants must make to acquire nutrients in Earth's subsurface. Plants allocate fixed carbon (C) to nutrient acquisition, but numerous variables, both physiological and environmental, may drive belowground plant C-resource allocation (Sasse et al. 2018). In rooting systems, plants allocate C to fine root production, rooting system exudates like organic acids and enzymes, and fungal symbionts that enhance nutrient mobility and uptake (Nadelhoffer and Raich 1992, Grayston et al. 1996, Jackson et al. 1997, Lynch and Ho 2005, Thorley et al. 2015). Each mechanism requires a different C investment, which could return a different nutritional benefit given the bioavailability of surrounding soil nutrient pools and the efficiency with which nutrients are released (Bunemann et al. 2011, Marschner and Rengel 2007). The utility of nutrient forms for plant productivity therefore may depend on the relative C tradeoffs required to access distinct nutrient pools, specifically those found in organic vs. mineral materials which are differentially distributed across soil profiles (Jobbagy and Jackson 2001, Lynch and Ho 2005). However, rooting systems' C allocation to different resource acquisition processes across soil depths, and their reliance on specific, depth-dependent nutrient stocks, are not well understood.

Further, rooting depths and nutrient distributions are currently subject to pressure from unprecedented global change agents. Agricultural and urban expansion, and cropland abandonment, all result in redistributed plant functional types with distinct root architectures, as well as a redistribution of nutrient forms (Amundson and Jenny 1991, Ellis et al. 2010, Haff 2010, Yoo et al. 2015). Additionally, rising atmospheric CO_2 levels alter the amount of C available to plants to make C for nutrient exchanges, with unknown consequences for future nutrient stocks (Cramer et al. 2001, Iversen 2010). These shifts may imbue lasting legacies deep in Earth's subsurface because of their influence on the rooting systems of plants (Billings et al. 2018). The time and depth extent to which C for nutrient economies reflect anthropogenic change is uncertain but important for understanding future nutrient sustainability and the fate of fixed C resulting from patterns of plant C allocation. Thus, clarifying the tradeoffs in rooting system strategies needed for nutrient acquisition in natural and anthropogenic systems would not only elucidate the sustainability of different nutrient forms, but would also refine our ability to make predictions about future drivers of belowground C cycling.

In response to these uncertainties about Anthropocene rooting systems, these studies characterize the role of rooting system C economies in nutrient-cycling phenomena and the extent to which contemporary rooting patterns, along with their C for nutrient tradeoffs, are a reflection of anthropogenic activity. I first examine rooting systems' nutrient tradeoffs across diverse forest ecosystems (Chapter 1) to characterize the nutritional strategies of rooting systems regardless of the presence of human activity. I then examine global patterns of contemporary and future rooting system distributions to contextualize the scope over which root-driven nutrient dynamics may be changing as a result of human activity (Chapter 2).

In this context, I probe the consequences of human induced changes to rooting systems by studying the rooting economies of specific, paired ecosystems with and without anthropogenic legacies (Chapter 3), and by analyzing the capacity for rooting systems to respond to their changing environments through a controlled greenhouse study (Chapter 4). In combination, these four studies demonstrate the ways rooting systems and rooting depth are changing in the presence of global anthropogenic changes, and the potential implications of contemporary and future rooting dynamics for nutrient availability and C cycling. Understanding these rooting system dynamics will be critical to sustaining ecosystem productivity and function as global change proceeds, thus helping foster sustainable biogeochemical cycles across Earth's surface through the activities of roots.

Chapter 1: Integrating decade and century timescales of root development with long-term soil development to understand terrestrial nutrient cycling

Hauser, E., Chorover, J., Cook, C.W., Markewitz, D., Rasmussen, C., Richter, D.D., & Billings, S.A. (2021). Integrating decade and century timescales of root development with long-term soil development to understand terrestrial nutrient cycling. *Ecosystems, in review*. Reproduced with permission from Springer Nature.

Abstract

Most terrestrial nutrient sources are hypothesized to shift in dominance from mineral- to organic matter (OM)-derived over millennia as soils weather. We investigated how overlaying this soil development framework with plant rooting dynamics that can feed back to soil development on relatively short timescales offers insight into ecosystem functioning. To test the hypothesis that root systems' nutrient economies mediate the nutritional importance of OM as mineral weathering proceeds, we paired litterfall decay experiments with soil mineralogical data from diverse forests across the Critical Zone (CZ) Observatory Network, USA. We demonstrate that sources of P shift from OM-bound stocks to minerals as the rooting zone expands during the transition from mid to late stages of forest growth. Plants thus develop root-driven nutritional strategies that do not necessarily rely most heavily on the dominant P form present in a system, typically driven by stage of soil development. Ecosystem P nutrition instead depends strongly on the interaction between dominant P form and rooting system development, particularly as it reflects past land use on both plants and soils. We use these findings to produce a novel framework of nutrient economics that highlights how rooting system growth and land use change can influence nutrient transformations and bioavailability in Earth's CZ.

Introduction

Nutrients vary in form and abundance in different environments (Porder and Ramachandran 2012), necessitating plant adaptations to a wide range of nutritional conditions (Reich and Oleksyn 2004; Ordoñez et al. 2009). Despite extensive research on plants and soils (Bormann and Likens 1967; Attiwill and Adams 1993; Vitousek et al. 1997; Lambers et al. 2008), we still lack clarity about the mechanisms by which plants obtain sufficient nutrition in diverse environmental conditions, and under what circumstances their nutrient sources change during ecosystem development. In the Anthropocene, understanding ecosystems' nutritional mechanisms becomes yet more elusive due to often-masked land use histories and patterns of perturbation that alter both soil nutrient stocks and the composition, developmental stage, and root system structure of the plants that access them. Improved understanding of these nutrient cycling phenomena will produce more accurate projections of future nutrient bioavailability, ecosystem productivity and vegetation contributions to global change.

Characterizing soil nutrient dynamics and associated vegetation response, regardless of past land use, has remained a challenge in part due to the contrasting timescales of soil development and vegetation response. Here, we use the term soil development to refer to the alterations in mineralogy and soil textural and hydrological properties that typically accompany pedogenesis (Vitousek and Farrington 1997; Chorover et al. 2007). We use the term rooting system development to refer to the proliferation of rooting systems – roots and their mycorrhizal symbionts – during vegetation growth (Doussan et al. 2003; Yan et al. 2006; Wang et al. 2009; Brearley 2011; Billings et al. 2018), the primary mechanism by which vegetation interacts with the developing soil system (Burghelea et al. 2015; Pierret et al. 2016; Billings et al. 2018; Dontsova et al. 2020). Most nutrients ultimately are sourced from minerals in rocks, which are renewed on long timescales via orogenic uplift (Carey et al. 2005; Vitousek et al. 2010) and released through the process of rock and mineral weathering (Drever 1994; Vitousek et al. 1997, Berner and Berner 2003; Richter and Markewitz 2000; Burghelea et al. 2015; Dontsova et al. 2020). These processes are typically measured over millennial time scales (Drever 1994; Brantley 2008; Ferrier et al. 2010). When taken up by plants, those nutrients are incorporated into biomass (OM) in aboveground and root tissues, and subsequently into soil organic matter (OM), comprising a nutrient source that is relatively abundant in more surficial soil horizons (Marschner and Rengel 2007; Gill and Finzi 2016). These resources cycle on annual to decadal time scales (Pedersen and Bille-Hansen 1999; Kavvadias et al. 2001). Models of soil development imply that the nutritional relevance of these OM-bound stocks must increase as soils develop over geologic timescales because weathering processes tend to decrease the abundance of mineral-bound P (Walker and Syers 1976).

Recent work highlighting interactions between soil and rooting system development (Lambers et al. 2008; Hobbie et al. 2015) underscores a need for a more detailed assessment of pathways of soil nutrient development. Indeed, vegetation is hardly a prisoner of its environment. Instead, rooting systems develop different nutrient acquisition strategies over time as a consequence of both changing nutrient demands and shifting abilities to tap into different resources, processes that feedback into soil development and subsequent nutrient stocks on comparatively short timescales (Lambers et al. 2008; Bardgett et al. 2014; Hauser et al. 2020). Plants allocate different amounts of fixed C to roots and mycorrhizae at different depths to cope with temporally shifting nutrient forms (Lambers 2008, Hauser et al. 2020; Peixoto et al. 2020), and plant uptake of nutrients can redistribute nutrient forms across the rooting zone, resulting in deep, mineral-bound nutrients being transferred to surface horizons in OM-bound forms on decadal timeframes (Jobbagy and Jackson 2001; Austin et al. 2018; Austin et al. 2020). All of these processes influence metrics of soil development such as mineralogy, soil structure and porosity (Jobbagy and Jackson 2001; Rasse et al. 2005; Pierret et al. 2016 ; Austin et al. 2018; Cui et al. 2019; Koop et al. 2020). Many of these strategies for acquiring nutrients are a consequence of increasing root extension over time, which typically enables plants to explore increasingly larger volumes of soil (Billings 1936; Doussan et al. 2003; Yan et al. 2006; Wang et al. 2008; Brearley 2011, Billings et al. 2018) and thus helps satisfy vegetation's increased nutrient demand as biomass accrues (Hasenmueller et al. 2017; Uhlig et al. 2017; Dawson et al. 2020). Combined, these processes suggest that models of nutrient partitioning require better integration with models of rooting dynamics because plant feedbacks, not just weathering-related soil development processes, influence the relative distribution of nutrient stocks. Such work would produce a novel framework describing the contribution of plant nutritional strategies to critical zone (CZ) development (i.e., development of whole ecosystems, including vegetation and regolith in tandem with each other; Jordan et al. 2001; Richter and Billings 2015). This effort seems especially apropos in the Anthropocene, when rooting depth distributions are undergoing rapid change due to land cover changes (Hauser et al. in review), with implications for weathering in diverse systems (Wen et al. 2020).

Here, we begin to characterize feedbacks between plants' nutrient exchanges and soil development to better understand how whole CZs' nutrient economies develop across diverse timescales. To do this, we estimate the potential dependence of vegetation on OM-bound nutrients relative to mineral-bound nutrients as these nutrient sources vary in dominance across forested CZs spanning continuums of soil and rooting system development. We focus our analyses on P because of its essential nature for vegetative growth (Penuelas et al. 2013, Jonard et al. 2015, Hou et al. 2020) and its presence in both rock minerals and OM (Vitousek et al. 1997). We discern the potential nutritional relevance of OM as mineral P stocks vary and discern the role of expanding rooting systems in soil developmental processes. We use these data to develop a novel conceptual model describing the development of CZ nutrient partitioning across timescales relevant to contemporary root expansion and soil development.

We hypothesized that any increase in the importance of OM-derived nutrients for forest nutrition across soil developmental stages is mediated by rooting systems and their changing interactions with soil minerals as trees age and rooting systems expand. We note that this hypothesis assumes that nutrient inputs from the atmosphere (Porder et al. 2007; Menge et al. 2012) remain constant over time. Over the timescales relevant to long-term soil development, we would expect that OM-bound nutrients must comprise a greater proportion of vegetation nutrient sources (Fig 1a). However, on shorter timescales, we hypothesize that root-mediated uplift of deep, mineral-bound nutrients made possible by the larger rooting volumes explored in later stages of forest vegetation development generates a relative decline in potential nutrient provisioning from OM as aging forests become increasingly able to tap into deep, mineral-bound nutrient stocks (Fig. 1b). If true, this hypothesis suggests that in forests composed of mature vegetation, mineral-bound P deep in the subsurface is relevant to vegetation nutrition even where weathering-induced P losses over time have been substantial. We test this hypothesis and demonstrate how different nutrient reservoirs play distinct roles in forest productivity as forests' rooting systems develop, as well as the influence those processes can have on soil development as both forest vegetation and soils change over diverse timescales.

Methods

Study Sites

We examined P cycling phenomena in multiple forests (Fig. 2a) comprising both the Critical Zone Observatory (CZO) and CZ Exploration Network (CZEN) sites that represent varied degrees of soil development and rooting system development (Fig. 2b). We use the chemical index of alteration (CIA) — a metric of weathering based on the relative prevalence of mineral Al, Na, Ca, and K calculated as oxides (Price and Vebel, 2003) — and soil taxonomic classification as soil development metrics (Fig. 2b), both of which offer an assessment of soil nutrient availability based on extent of soil weathering and OM inputs. Although plant species and thus rooting architecture (Freschet et al. 2017) and foliar and litterfall nutrient concentrations (Hobbie et al. 2006; Hobbie 2015) differ across these sites, our focus is on root proliferation over time in diverse forests and the distinct strategies roots develop for obtaining nutrients. Our approach thus integrates these species differences.

The site with the most well-developed soils is the Calhoun CZO, South Carolina, USA, which supports hardwood forests with ~100-200 y old trees and re-growing pine forests with ~50-90 y old trees on highly weathered Ultisols derived from granitic gneiss bedrock (Bacon et al. 2012). The dominant vegetation at the hardwood sites is comprised of *Quercus alba, Liquidambar styraciflua, Liriodendron tulipifera, Q. rubra,* and multiple *Carya spp.* The pine sites are dominated by *Pinus taeda* and *P. echinata*. The Calhoun CZO ranges in elevation from 134 to 190 m, has a mean annual precipitation (MAP) of 1250 mm y^{-1} and mean annual temperature (MAT) of 16°C (Critical Zone Observatories 2020a).

Sites with moderately developed Inceptisols and more highly weathered Ultisols support middle-aged, ~95-110 y old hardwood forests at the Shale Hills CZO, Pennsylvania. Sites here range in elevation from 256 to 310 m and have a MAP of 1050 mm y^{-1} and MAT of 9.5°C

(Critical Zone Observatories 2020b). Within the Shale Hills CZO, there are two subsites with differing underling lithologies: Rose Hill Shale regolith at the Shale Hills watershed site, and sandstone at the Garner Run watershed site. Vegetation at these sites is dominated by multiple *Quercus spp., Carya spp.* and *Pinus spp.*

At the Catalina CZO, Arizona, USA, forests grow on Mollisols and Inceptisols formed predominantly from granite in the high elevation Marshall Gulch (2284-2634 m) and mid-elevation Oracle Ridge (2064 to 2388 m) sites. Marshall Gulch forests are dominantly ponderosa pine (*Pinus ponderosa*) and white fir (*Abies concolor*), while Oracle Ridge sites span a vegetation transition from oak-dominated to the pinyon-juniper community type (dominated by *Pinus* and *Juniperus spp*.) to ponderosa pine stands. The MAP values for the Marshall Gulch and Oracle Ridge sites are 940 mm y^{-1} and 840 mm y^{-1}, and MAT values are 10.4°C and 11.9°C, respectively (Critical Zone Observatories 2020c). These soils support relatively old, mixed conifer vegetation averaging ~150 y in age.

Finally, the University of Kansas Field Station (KUFS) forest is supported by Mollisols formed from glacial till and loess that are relatively rich in both mineral nutrients and organic matter (Klopfenstein et al. 2015, Hirmas and Mandel 2017). This forest is composed of a ~90 y old oak-hickory forest growing on former agricultural lands that had replaced the tallgrass prairies soon after European colonization. In regions of the forest that experienced particularly heavy degradation under agricultural use, Eastern redcedar (*Juniperus virginiana*) is also a dominant species. Elevation at KUFS is 335 m, MAP is 940 mm y^{-1} and MAT is 13°C (Kansas Biological Survey 2020).

Rooting system development time

We relied on site histories and tree surveys to estimate the amount of time roots at each site could have been exploring the soil profiles. This metric, which we term rooting system development time, is one possible independent variable for examining the importance of different nutrient forms to forest nutrition. Each site's unique history prompted individual site assessments to develop age estimates representative of the time over which roots similar to those found today have explored their soil profiles. At the Calhoun pine forests, KUFS forests, and the Shale Hills and Garner Run forests, known histories of recent land use and tree reestablishment after stand-replacing disturbances determined the amount of time roots of the contemporary vegetation may have explored soils at these locations. Both Calhoun pine and KUFS forests are reestablishing after agricultural use ~70 and ~80 y ago respectively (Richter and Markewitz 2001; Fitch et al. 2001). Shale Hills and Garner Run sites were subject to widespread logging at the beginning of the 20th century, such that roots in these forests have had ~110 and ~90 y to explore, respectively (Li et al. 2018). The Catalina and Calhoun hardwood forests represent later successional stages, comprised of self-replacing tree species (Whittaker 1953), suggesting that these forest soils have been explored by rooting systems similar to contemporary roots for longer than the age of contemporary trees might suggest. We employ ages of the oldest sampled trees at these sites as a conservative estimate of the amount of time these soils have been explored continually by roots similar to those that exist today. At Catalina, this is ~150 y and at Calhoun, ~200 y (Richter and Markewitz 2001; Iniguez et al. 2016). Our rooting system development metric is broad, encompassing both fine and coarse roots, and is representative of a potential—the maximum possible time for contemporary roots to have explored these soils. Though changes in species over time in these forests likely have driven changes in root architectures, we consider time since perturbation to be a key driver of rooting system development and the volume of soil roots can access, given that roots proliferate more extensively with age (Billings 1936; Zangaro *et al.*, 2008; Knops and Bradley 2009; Devine *et al.* 2011; Yuan & Chen, 2012; Sun *et al.*, 2015).

Data Collection

Characterizing forest P nutrition status

We approximated annual vegetation P uptake at each site by estimating net primary productivity (NPP) and converting those values to P demand (NPP_p) via estimates of vegetation stoichiometry. We used annual litterfall biomass (g m^{2} y^{-1}) to estimate NPP (g C $m^{2}y^{-1}$) given its direct linkage to yearly ecosystem NPP (Matthews 1997). To do this, we converted leaf mass to leaf C approximating leaves to be 48% C (Bowden et al. 1992). We converted these litterfall-based NPP estimates to NPP_p using C:P in leaves collected from each site, producing values of potential P uptake that reflect the varied P demands of our study sites. Senesced leaf P concentrations were assessed using the methods detailed below. Our approach relies on the assumption that the linkage between litterfall rates and NPP is robust across all forests (Matthews 1997).

We estimated total soil P using bulk elemental data (Jin et al. 2010; Holleran 2013; Austin & Schroeder 2019; Brantley 2019; National Ecological Observatory Network 2020a). We transformed P_2O_2 and %P estimates into P contents of each sampled horizon using bulk density distributions (Richter et al. 1994; Herndon 2012; Holleran 2013; Bacon 2014; Brantley 2019; National Ecological Observatory 2020a). We summed P contents across horizons to the depth approximating 95% rooting biomass to estimate the absolute P content of a square meter soil column in the rooting zone. We estimated the depth at which 95% of the rooting biomass is attained (D95) using rooting distributions from soil profiles sampled at each site (Holleran 2013; Li et al. 2018; Eissenstat 2019; Billings et al. 2020; National Ecological Observatory Network

2020b). Though roots growing below D95 can penetrate into saprolite or bedrock (Hasenmueller et al. 2017) our use of D95 captures most root activity and permits comparison of an analogous metric across sites. Though clay-rich horizons are well-represented at some of these sites, to our knowledge there are no impediments to proliferation of roots as severe as hardpans at any forest studied. We anticipate that the D95 soil depth captures most of the soil volume over which roots may interact with soil P distributions.

P in Leaf Biomass

We analyzed the P concentration of leaves from each site to generate NPP estimates and to assess leaf P release rates. Leaves were dried at 60°C for at least 3 days and ground using a mortar and pestle to pass through a 2 mm mesh sieve before shipment to the Kansas State Soil Testing Lab. There, leaves were further processed using salicylic-sulfuric acid digestion before analysis for bulk elemental concentration using inductively coupled plasma – optical emission spectroscopy (ICP-OES, Varian 720-ES, Palo Alto, USA).

Maximum potential organic P recycling

To examine comparable potentials for annual OM inputs to the soil that could meet plant P uptake in these forests, we developed a metric of the maximum release rate of organic P (P_o) from the OM (OM_P) produced in a year in each forest, normalized by annual plant P uptake (OM_P:NPP_P). To estimate sites' potential annual P_o release rates, we conducted decomposition experiments using leaves collected from each study site. We compare P release rates across temperature and moisture conditions relevant for each forest such that rates represent decomposition rates realistic to each site. Conducting these decomposition experiments in the lab instead of the field permitted us to specifically examine annual P release potential without influence from P remaining in soil OM, whether particulate or mineral associated, from previous seasons. Additionally, the lab-oriented design made for greater ease of cross-site work, given that sites were widely dispersed geographically. Though our design did not allow litter exposure to soil macrofauna, which can influence soil P distributions (Chapuis-Lardy et al. 2011), we included microbes native to each site (see below) to aid decomposition.

At the Catalina and KUFS sites, we deployed 10 to 12 litter baskets in fall 2016 and spring 2017, respectively, for leaf litter collection over the course of the following growing season. At KUFS, litter collection for NPP estimation has continued for 3 additional seasons, but only leaves from 2017 were used for incubations. Previously collected, senesced leaves were available from the prior 2015 and 2016 growing seasons at the Calhoun and Shale Hills CZOs. Upon collection, leaves were dried at 60°C until a constant weight before grinding with a Wiley mill.

To inoculate these oven-dried, senesced leaves with microbes able to induce decay, we added a small amount of freshly collected, senesced leaves from each site to their respective dried counterpart. During the spring of 2017, we collected a small sample of freshly fallen leaves from each site, which we froze upon return to University of Kansas labs. We thawed these samples, roughly chopped them with a knife and mixed a small amount with the dried litter. We distributed ~0.7 dry g of the mixed litter into six small permeable bags per litter collection trap and placed the bags into a glass jar filled partially with marbles to keep the bags well drained. This resulted in at least six jars per site containing six bags each. We then incubated leaves aerobically in site-relevant conditions (Table 1). We periodically watered the bags in quantities and frequencies mimicking site-specific growing season rainfall (Table 1). To mimic the bimodal precipitation regime at the Catalina CZO, we performed two shorter incubations to approximate the amount of decomposition possible over a full year's time course at this site. We periodically subsampled the litter bags and collected the water that had leached through them during rain-mimicking events. These subsamples allowed us to examine P released during OM decay and not immobilized in microbial biomass over a time course representative of each site's growing season.

At the beginning and end of the incubation, we removed one of the subsample bags and analyzed litterfall P concentration (described above). Using litterfall mass over time, we calculated P content of each leaf subsample over time. Differences between initial and final P contents in decaying leaves per unit time provide estimates of potential annual P_o release (the numerator in OM_P:NPP_P). We assume that the potential P release from OM already present in the upper soil profile is lower than the rates achieved with fresh litter, so these estimates represent a maximum potential P release from annual OM additions. Our incubation approach does not permit knowledge of absolute P release from OM decay throughout these soil profiles. However, it provides comparable information about each location's annual potential to offer P from OM in plant available forms.

Metrics of soil mineral P status

Using previously collected soil mineralogical data (Jin et al. 2010; Holleran 2013; Austin & Schroeder 2019; Brantley 2019; National Ecological Observatory Network 2020a), we calculated soil weathering indices to numerically characterize the mineral nutrient status of each site, as well as biotic contributions to mineral distributions. We focused on tau_P (τ_P)—a metric describing depletion of P in the soil profile relative to the soil parent material (Ruxton et al. 1968, Price and Vebel 2003, Oh et al. 2007). Traditionally, τ relies on comparing a soil sample from a specified depth in the soil profile with a reference material representing the parent lithology; a more negative value of τ indicates that the soil examined has been depleted in the element of interest relative to the parent material reference, while a positive value indicates elemental enrichment (Brimhall 1987).

Calculating τ relies on measurements of a relatively immobile element as a reference to which the more mobile element of interest can be compared. Here, we used Zr as the element with limited mobility. Calculations of τ also vary depending on the soil horizon of interest and potential uncertainty of parent material. We calculated τ_P of the rooting zone given our interest in vegetation influences on soil development metrics, using the ratio of P remaining in the soil surface horizon sampled at each site relative to P at the soil depth where roots have reached ~95% biomass. We thus replaced parent rock P in the traditional calculations of τ with P of soil where roots are rare, achieving a metric of P depletion across the depth of soil inhabited by the largest proportion of plant roots. We determined the depth of ~95% rooting biomass for the rooting front components in each equation from data collected during prior soil sampling at each site (Holleran 2013; Li et al. 2018; Eissenstat 2019; Billings et al. 2020; National Ecological Observatory Network 2020b). We thus calculate τ_P as follows:

$$\tau_P = \frac{P_t^S * Zr^{RF}}{P_t^{RF} * Zr^S} - 1 \qquad \text{(Eqn. 1)}$$

where P_t^S is total P (mg kg^{-1}) in the uppermost sampled soil horizon, Zr^{RF} is the total Zr (mg kg^{-1}) at the approximated rooting front where roots reach ~95% rooting biomass, Zr^S is the Zr (mg kg^{-1}) in the uppermost soil horizon, and P_t^{RF} is total P (mg kg^{-1}) at the rooting front.

While this metric may not explicitly reveal the complexities of any P additions and losses, especially when calculated across the rooted zone, we modified this metric in a way that offers a means of exploring the time-integrated ecosystem dynamics of P_i and P_o separately. We can further focus our analysis on inorganic P (τ_{Pi}) by subtracting estimates of P_o from total soil P values before implementing equation 1. The τ_{Pi} metric thus reflects changes over depth specific to mineral-bound P. τ_{Pi}, therefore, allows us to estimate the changes to inorganic, mineral-bound P across the depth of the root zone. This produces the following equation:

$$\tau_{Pi} = \frac{P_i^S * Zr^{RF}}{P_i^{RF} * Zr^S} - 1 \qquad \text{(Eqn. 2)}$$

where P_i^S is inorganic P in surface soils estimated by subtracting organic P estimates from total P estimates, and P_i^{RF} is inorganic P at the rooting front estimated by the same method. This allows us to examine the P status of inorganic, mineral-bound P separate from organic P. We generated estimates of soil P_o from soil organic C depth distributions available for each site (Rasmussen et al. 2008; Andrews et al. 2011; Holleran 2013; Hasenmueller et al. 2017; Brantley 2019; Billings et al. 2020; National Ecological Observatory 2020c), and OM C:P ratios estimated from sites' litter data, the most site relevant C:P ratio we have available for initial inputs of OM to soil. There is some evidence that the C:P in roots, a primary soil C source, is comparable to leaf litter C:P, lending greater confidence to use of litter C:P ratios (Zechmeister-Boltenstern et al. 2015). However, this method may underestimate P_o given recent demonstrations that OM in mineral soil conserves P_o more effectively than organic C (Spohn 2020). The difference between τ_P and τ_{Pi} provides us with an estimate of τ_{Po}:

$$\tau_P - \tau_{Pi} = \tau_{Po} \qquad \text{(Eqn. 3)}$$

The τ_{Po} metric helps us understand the role of P_o in the P status of root zone soils.

Statistical Analyses

We first characterized P demand and P availability across forest sites to better understand the outcomes of our hypothesized relationships. To do this, we regressed NPP_P and site leaf P concentrations on our estimates of rooting zone P content. These relationships act as an indicator of relative P limitation across the forests examined in our study and characterize forest productivity across a variety of soil conditions.

To test whether our estimates of potential annual OM provision of P could meet a greater proportion of forests' NPP_P demands where soils are more depleted in mineral-bound P, we performed regression analyses between the OM_P:NPP_P ratio and $\tau_{Pi,}$ and τ_{Po} metrics. We log transformed the OM_P:NPP_P ratio and τ_{Pi} to meet the assumption of normally distributed residuals. Other metrics did not require transformation to meet assumptions. These comparisons discern the maximum capacity for OM recycling to sustain forest P uptake in soils of different weathering statuses and, in the case of τ_{Po}, the ways in which soil and stand development display feedbacks as a result of rooting systems driving P_o distributions.

To determine whether OM_P varied in nutritional relevance with forest development, we performed a linear regression between stand age and the OM_P:NPP_P metric. We tested for outliers using Cook's Distance and calculated the regression both with and without outlier points. We also performed a one-way ANOVA using stand ages as categorical variables driving the OM_P:NPP_P metric to discern whether we observed distinct groupings of forests based on their reliance on OM- vs. mineral-based P. We followed t-tests with post hoc Tukey tests to discern which forests exhibited similar OM_P:NPP_P responses, and what response patterns emerged as a function of forest age. Data followed the assumptions of normally distributed residuals.

To assess the degree to which vegetation age was associated with patterns of soil P forms, we analyzed the relationship between stand age and τ_{Po}. We fitted linear and non-linear curves to the data and selected the best fit via AIC values. In all described analyses, we report every meaningful trend, which embraces P-values <0.1 given the difficulties inherent in seeking appropriate ecosystem-scale site replicates, as well as the difficulty of collecting comparable ecological data across dispersed sites (Filion et al. 2000; Oren et al. 2001; Bernacchi and Morgan 2005; Amrhein et al. 2019). Analyses were performed in RStudio v. 1.0.153 (RStudio Team 2017).

Results

Vegetation P demand estimated via NPP (NPP_P) was positively correlated with total rooting zone P ($P = 0.013$, $r^2 = 0.7$, Fig. 3a). Soil depths by which roots have achieved 95% of their biomass, which were used to approximate the depth of most of the rooted zone and determine the rooting front (RF) terms in τ_P calculations, ranged from 3.2 to 0.6 m (Table 1). Leaf [P], a measure of vegetation P status, was positively correlated with total P of these rooted zones ($P = 0.07$, $r^2 = 0.75$, Fig. 3b).

P release from OM decay, used to calculate our metric of OM recycling, ranged from 0.085 to 0.31 gP $m^{-2} y^{-1}$ across sites (Table 1). The relationship between $OM_P:NPP_P$ and τ_{Pi} was best represented by an exponential decay curve (AIC = 1.146507), reflecting its steeply negative slope where soils have negative τ_{Pi} values. The AIC values for other tested relationships, including a linear relationship, ranged from 1.65 to 16.22. Where sites were more depleted in inorganic P across the rooting zone, OM recycling exhibited a near-significant rise, suggesting the potential to meet a greater proportion of annual forest P demand ($P = 0.07$, $r^2 = 0.41$ Fig. 4). Given the relatively few forests available to contribute data, the marginally significant linear relationship they exhibit, and the better fit of the exponential equation, the fitted curved hints at increased nutritional relevance of OM-bound P where mineral P is depleted.

Stands that experienced longer duration of exploration by contemporary roots displayed greater contributions of P_o to total soil P enrichment (i.e., higher values of τ_{Po}). Comparatively younger root systems exhibited less enrichment of soil P by P_o (Fig. 5a). However, with one exception, potential P release from OM decay was relatively high for less well-developed rooting systems and comparatively low where roots had been established for a longer time (Fig. 5b, P = 0.015, r^2 = 0.76). The exception to this finding is the KUFS forest (Cook's Distance = 1.16), where the potential for OM P to meet estimated NPP_P demands is lower than the other similarly aged forests. Compared to the ~80 y old Calhoun pine forests and ~95 y old Garner Run forests, ~81 y old KUFS forests displayed 0.64 and 0.46 percent less P provision from OM as a proportion of NPP_P demands (P <0.002), respectively. Compared to ~110 y old Shale Hills forests, KUFS forest's OM_P provision was 21% less (P = 0.08).

Discussion

Our findings demonstrate the interwoven nature of vegetation and soil dynamics that calls for greater consideration of decadal-scale rooting system processes in metrics of soil development. When examined solely in terms of mineral P status, one metric of long-term pedogenic time (Fig. 1a, x-axis), our data are consistent with the patterns of nutrient bioavailability we might expect under traditional models of soil development (Walker and Syers 1976, Vitousek et al. 1997; Izquierdo et al. 2013). We observed the highest potential release rate of OM-bound P where soils were comparatively mineral-P depauperate and the lowest potential release rate of OM-bound P at sites with soils relatively rich in mineral-bound P (Fig. 4). Within the forests studied, where P availability is dominated by either OM- or mineral-bound P, vegetation rooting systems leverage a nutrient strategy based on the P form that is most bioavailable, a feature that varies across geologic timescales (Walker and Syers 1976, Crews et al. 1995, Hauser et al. 2020). However, when we examine our findings through the decadal-scale lens of rooting system development, we observe deviations from what we might expect for the role of OM- vs. mineral-bound P in forest nutrition if we only considered soil weathering and P losses from the entire system over geologic time (Walker and Syers 1976).

The relevance of OM-derived nutrients for forest nutrition during the course of CZ development is a reflection not just of soil elemental losses, but also of the rooting systems that develop during decades to centuries of vegetation growth. Throughout this shorter timeframe, nutrient demands increase (Mou et al. 1993; Rode 1993; Vitousek et al. 2010) and rooting systems expand to explore increasing soil volumes for nutrients (Billings 1936; Zangaro et al. 2008; Knops and Bradley 2009; Yuan and Chen 2012; Sun et al. 2015). We cannot know the functionality of all deep roots (Nippert et al. 2012; Nippert and Holdo 2015), but as roots reach deeper into soil profiles over the lifetime of forest communities, they have the potential to increasingly access deep, mineral-bound nutrients. These dynamics can alter soil profiles in ways that feed back into the distribution of mineral- and OM-bound nutrient stocks, sometimes producing unanticipated trajectories of soil development. Vegetation mediates this shift to enhanced mineral dominance through strategic allocation of C to resource exchanges that vary over vegetation's lifespan (Marschner and Rengel 2007; Lambers et al. 2008; Bardgett et al. 2014; Hauser et al. 2020), thereby contributing to distinct soil developmental patterns on root-growth driven timeframes.

The results from the forests we examined suggest a root-promoted shift in the relative dominance from organic to mineral nutrient sources as forests age (Fig. 5), regardless of the degree of development of the underlying substrate. This finding is in contrast to some contemporary conceptual models (Lambers et al. 2008; Phillips et al. 2013), which suggest that forests trend toward relatively OM-dominant supplies over time. We observed mineral P depletion where the bulk of roots are present in the youngest root systems (Fig. 4, sites with $\tau_{Pi} < 0$) as well as relatively high P_o input rates (Table 1), suggesting that aboveground biomass has accumulated sufficiently to provide a readily available OM-rich nutrient pool to the upper soil horizons (Crews et al. 1995; Balogh-Brunstad et al. 2008). However, root systems in these still comparatively young forests likely have relatively limited proliferation below the OM-rich horizons (Dupouey et al. 2002; Mobley et al. 2013; Billings et al. 2018; Hauser et al. 2020). This prompts an OM-dominated nutrient economy, apparently regardless of the amount of soil developmental time, as evidenced by the high potential for OM to provide P to vegetation in multiple forests where rooting systems have been developing for ~80 to 110 y, even across a diversity of soil developmental stages (Fig. 4, values below 0 on the x axis, Fig. 5a).

As rooting system development proceeds, a greater abundance of roots extends past the organic rich horizons to the weathering front (Knops and Bradley 2009; Yuan and Chen 2012; Pierret et al. 2016; Billings et al. 2018), where they are in contact with less weathered mineral surfaces (Brantley et al. 2012; Hasenmueller et al. 2017). Deep roots mine this mineral nutrient source, in part because mineral-bound P is less C expensive to liberate than OM-bound P (Smith 1976; Hauser et al. 2020). This prompts the observed shift to a mineral focused P supply at these depths with decreased potential P provision from OM in sites with the oldest forest stands despite varied degrees of underlying substrate development (Fig. 2b & 5). Thus, forests with older vegetation and well-developed rooting systems appear able to develop a mineral-focused P economy even where soils are well-developed and, presumably, primary mineral P is depleted in the rooted zone. Primary mineral P stocks traditionally are not strongly implicated in the P nutrition of highly-developed CZs due to their oft-observed decline across the soil development continuum (Walker and Syers 1976, Fig. 6). We thus expand Walker and Syers' idea to incorporate root development over time, demonstrating that even where mineral bound P is scarce, if present, it is likely still relevant to ecosystem P nutrition (Fig 6, red line 2). Note that none of our sites are true endmembers of Walker and Syers' (1976) concept (i.e., Oxisols), so there may be an increase in the nutritional relevance of OM-bound P in very developed systems that we did not observe in this study (Fig. 6, red line 3). Our observations across the studied forests lend support to our proposed addition to Walker and Syers' soil development model and our hypothesis that rooting systems drive forest nutrient economies despite a soil's status within the soil development continuum (Fig. 6).

A primary mechanism underlying the nutritional transition from OM to mineral forms likely is the resource tradeoff that rooting systems face when confronted with different forms of the same nutrient. Perhaps as a consequence of relatively low C cost for acquisition of mineral-bound nutrients (Smith 1976; Hauser et al. 2020), we demonstrate that vegetation may preferentially utilize mineral-bound forms. The KUFS forest, an outlier to many of the patterns detailed above (Fig. 4 & Fig. 5b), highlights the potential for roots to economize C for P. In contrast to other similarly-aged systems in this study, P provision from OM recycling does not appear able to provide a large portion of KUFS trees' nutrient demands (Figs. 4 & 5b). These lands have been subjected to human activity linked to altered metrics of soil development (Amundson and Jenny 1991; Haff 2010; Yoo et al. 2015) and vegetation development (Ellis et al. 2010; Mcdowell et al. 2020), and likely have generated novel nutrient depth distributions and truncated rooting systems (Billings et al. 2018; Hauser et al. 2020). Both KUFS soil P contents and leaf [P] values are high (Fig. 2), suggesting a lack of P limitation (Ordoñez et al. 2009; Hou et al. 2020). The soils likely contain mineral P from past fertilizer applications and are rich in organic P as well due to their tallgrass prairie legacy (Balesdent et al. 1988). This hints that when both nutrient forms are readily available, roots preferentially implement a less C-intensive mineral-based P economy. It is beyond the scope of this study to fully test a hypothesis focused on land use history, nutrient economies, and soil development. However, given pervasive, global-scale land use changes and fertilization of organic-rich soils that once supported native grasslands (Brye and Pirani 2005; Ellis et al. 2010), shifts of ecosystems toward selective, mineral-based economies, such as that observed at the KUFS forest, may occur in many systems, with yet unseen soil development feedbacks.

Conclusion

This work demonstrates the biologically-derived complexities that result in nuanced but important deviations from projections of dominant nutrient sources that emerge from soil development frameworks such as that proposed by Walker and Syers (1976). Specifically, we highlight how vegetation growth through time and land use history can influence whether hypothesized patterns of dominant nutrient sources over soil developmental timeframes are realized in Earth's critical zone. The feedbacks we observed between geologic and biologic drivers of soil development demonstrate the strength of biotic processes in geologic phenomena that occur many meters below Earth's surface. These processes sometimes result in the dominance of unanticipated nutrient sources for forest vegetation and misleading metrics of elemental depth distributions in soils that can mask the degree of soil development. In the Anthropocene, human alterations to both plants and soils produce novel alterations to soil developmental patterns through their influence on rooting system economies. Thus, incorporating rooting system development into concepts of plant nutritional strategies across soil developmental time in the context of anthropogenic change will help us more accurately forecast regolith nutrient dynamics and forest C sink strength.

Tables

Table 1. Data used for calculation of potential P provision and τ_P ratios. These include release rate of P from OM (OM_P) as determined by litter incubations, estimates of P uptake to meet the demands of NPP at each site, and the depth to 95% rooting biomass used to determine the soil sample depths to use for calculating τ_P. We also include an estimate of depth to bedrock at each site for comparison of potential soil volume accessible to roots at each location. The derivation of table values, as well as τ_P values and an explanation of error estimates are outlined in the main text (Methods section) and in captions for figures associated with each dataset.

Site	OM_P (g_P m^{-2} y^{-1})	NPP_P (g_P m^{-2} y^{-1})	Depth to 95% Rooting Biomass (m)	Depth to Bedrock (m)
Calhoun Hardwood	0.19	0.42	3.2	5 – 40
Calhoun Pine	0.20	0.20	2.9	5 – 40
Shale Hills	0.16	0.26	0.5	<0.25 (ridge top) to >2 (valley floor)
Garner Run	0.31	0.37	1.2	0.7 (ridge top) to 1.7 (valley floor)
Marshall Gulch	0.14	0.26	0.6	0.7 – 1.8
Oracle Ridge	0.09	0.16	0.6	0.7 – 1.8
KU Field Station	0.24	0.65	2.0	0.5 – 2

Table 2. Summary of incubation characteristics for each study site. Range of water applied indicates the minimum and maximum amount of water added to incubation jars per forest floor area represented by litterfall in jars. The amount of water applied at each time point was based on the average monthly precipitation for each site corresponding to the month of the incubation. See text for details.

Site	Incubation Length (d)	Incubation Temp (∞C)	Range of water applied (mL cm^{-2})	Source
Calhoun CZO	212	21.5	7.5 – 11	https://criticalzone.org/calhoun/infrastructure/field-areas-calhoun/
Catalina Jemez CZO Marshall Gulch	Incubation 1: 115 Incubation 2: 117	Incubation 1: 7 Incubation 2: 7	0.8 – 15.7	https://criticalzone.org/catalina-jemez/infrastructure/field-areas-catalina-jemez/
Catalina Jemez CZO Oracle Ridge	Incubation 1: 115 Incubation 2: 117	Incubation 1: 10 Incubation 2: 10	0.7 – 10.9	https://criticalzone.org/catalina-jemez/infrastructure/field-areas-catalina-jemez/
University of Kansas Field Station	185	21.5	8.8 – 13.3	https://biosurvey.ku.edu/sites/kbs.drupal.ku.edu/files/docs/Climate%20Synopsis.pdf
Shale Hills CZO	154	19.3	8.8 – 10.4	https://criticalzone.org/shale-hills/infrastructure/
Garner Run	154	19.3	8.8 – 10.4	https://www.hydroshare.org/resource/9535cbe97d5843a788fc7648de39a6e5/

Figures

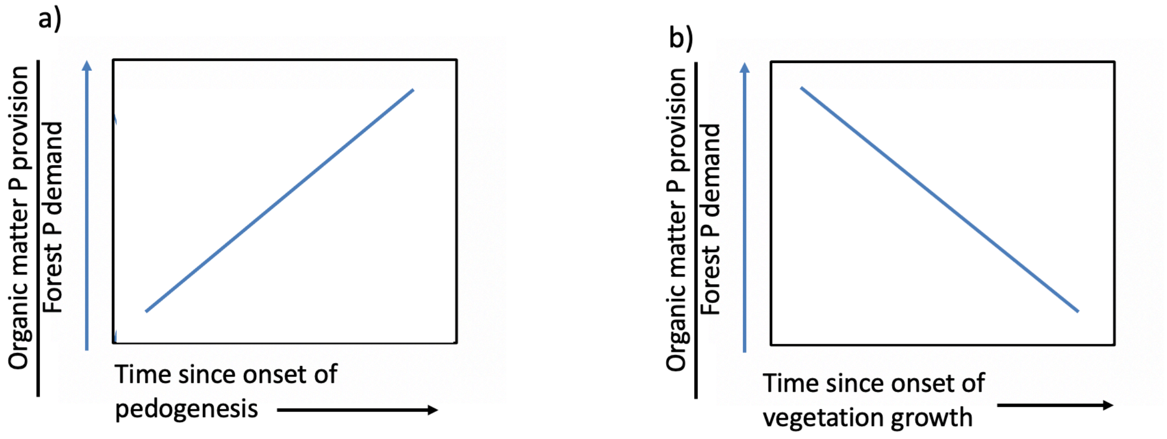

Figure 1. Predicted relationship between the potential provision of nutrients from OM as pedogenesis proceeds, as a consequence of Walker and Syers' proposed model (1976; a). In contrast, we offer (b), a hypothesized relationship between provision of nutrients from OM as vegetation rooting systems develop, reflecting how rooting system interactions with soil development may lead to increasing provision of nutrients from minerals and reduced potential forest P provision from OM as trees age.

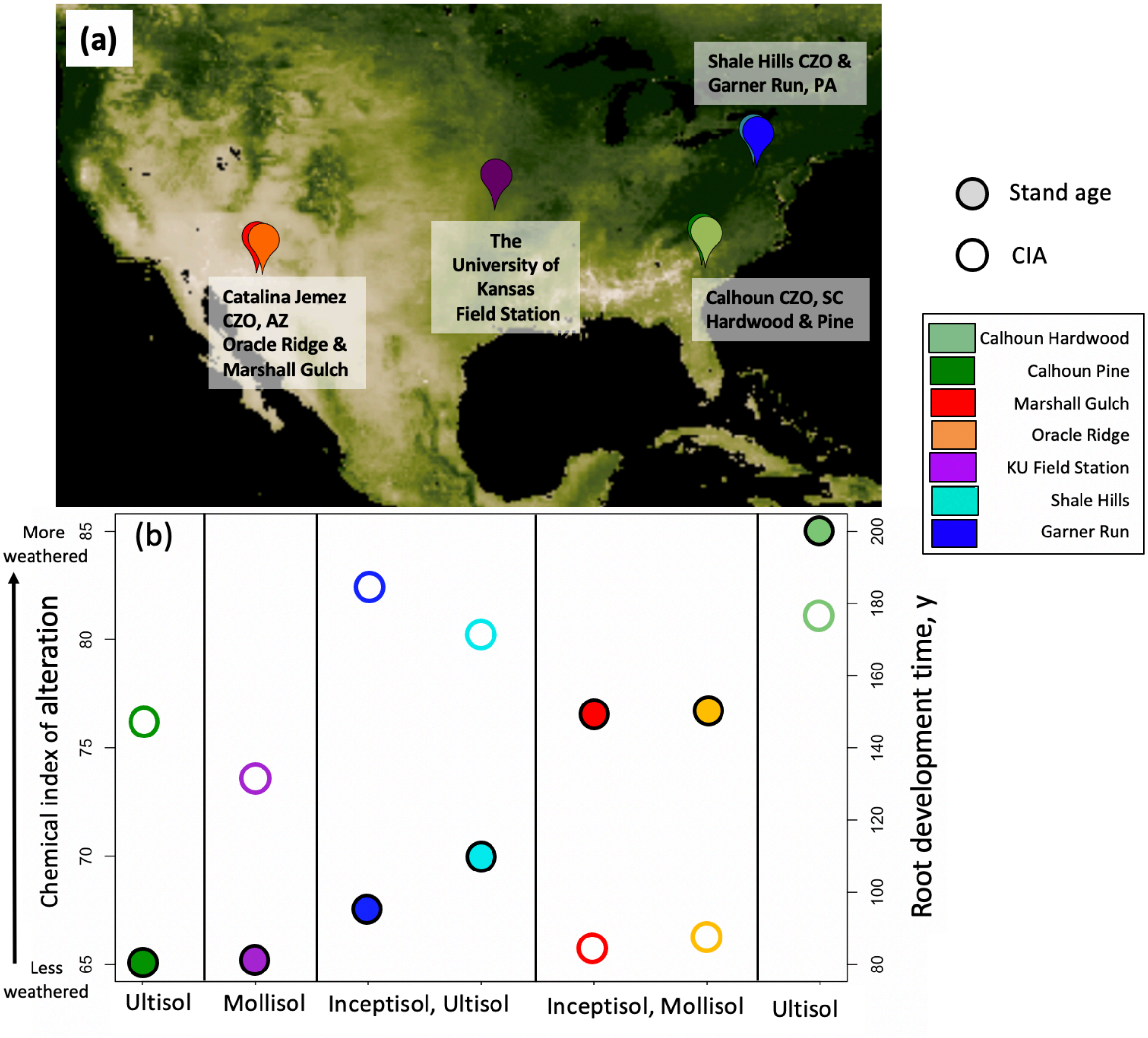

Figure 2. Field sites across the Critical Zone Exploratory Network (a) representing a gradient of soil mineral development as estimated by the Chemical Index of Alteration (CIA)—a metric of Al, K, Ca, and Na oxide depletion—and soil order (b, open circles). Lower CIA values indicate a less weathered material and higher CIA values indicate stronger weathering and elemental depletion. These sites also represent a gradient of rooting system development (b, right y axis, filled circles) overlaying the diverse soil environments.

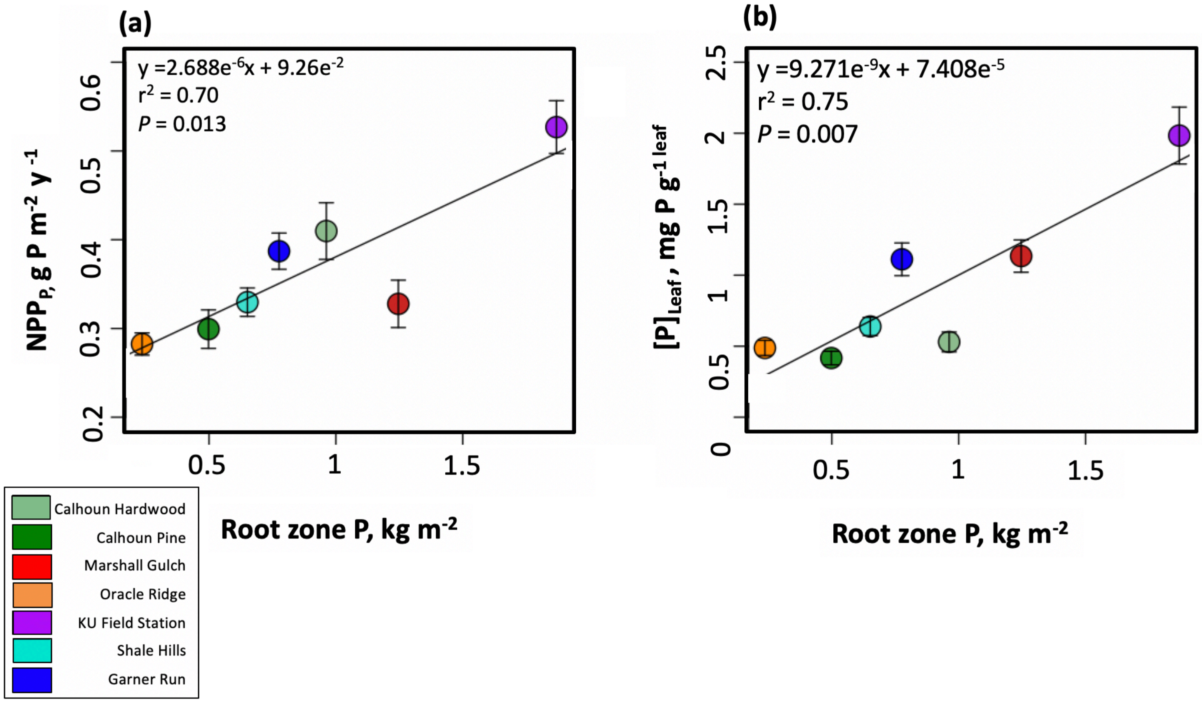

Figure 3. Characterization of vegetation growth characteristics in relation to soil P content. Total P content of a square meter soil profile summed to the depth of ~95% rooting biomass, termed root zone P, displayed positive relationships with both (a) P demand of net primary productivity (NPP_P) estimated from annual leaf litter production *(P* = 0.013, r^2= 0.7) and (b) leaf [P] in forest trees at each study site (*P* = 0.007, r^2= 0.75). Error bars represent one standard error from the mean.

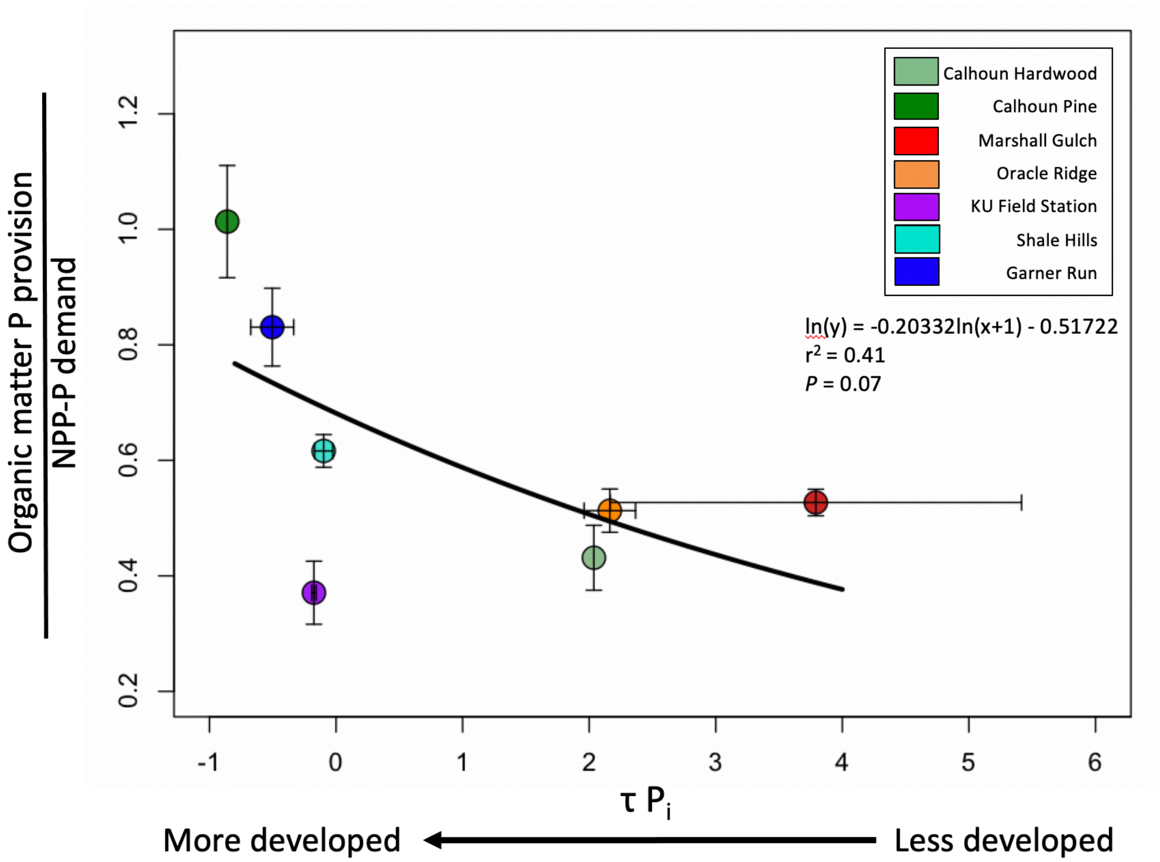

Figure 4. Estimated proportion of each forest's annual P demand (g P m^{-2} y^{-1}) potentially provided by annual organic matter decay (g P m^{-2} y^{-1}) compared to depletion of inorganic P (τ_{Pi}) across the rooting zone. Negative τ_{Pi} values represent P depletion across the rooting zone, while positive values indicate P enrichment. Log-transformed data display a marginally significant correlation ($P = 0.07$, $r^2 = 0.41$) and are best represented by an exponential decay curve, suggesting a non-linear pattern to organic matter nutrient provision across stages of soil development. Error bars represent one standard error from the mean, calculated from incubation and soil sampling replicates. Walker and Syers (1976) posited that mineral-bound P becomes increasingly depleted in soils with time; if so, greater OM-P provision where soil is more developed might be expected, similar to the trend revealed here.

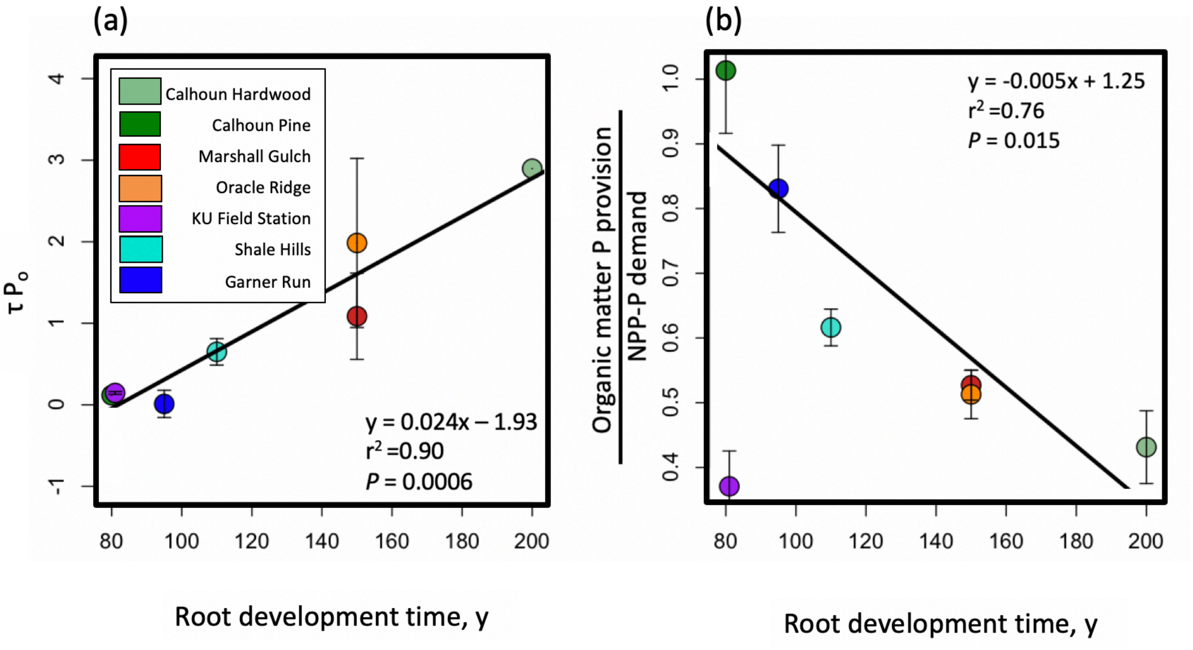

Figure 5. Demonstrations of the role of forest nutrient economies in subsurface development. (a) Organic P enrichment (τ_{Po}) across the rooted profile vs. root development time (y), indicating the extent to which OM stocks and recycling in upper soils can mask evidence of soil P_i loss. (b) Estimated proportion of each forest's annual P demand (g P m^{-2} y^{-1}) potentially provided by annual OM decay (g P m^{-2} y^{-1}) vs. root development time. Statistics are calculated after removal of the KUFS site, which is a significant outlier (Cook's Distance = 1.16; see discussion in text for details). Error bars in both plots represent one standard error from the mean, calculated from incubation replicates.

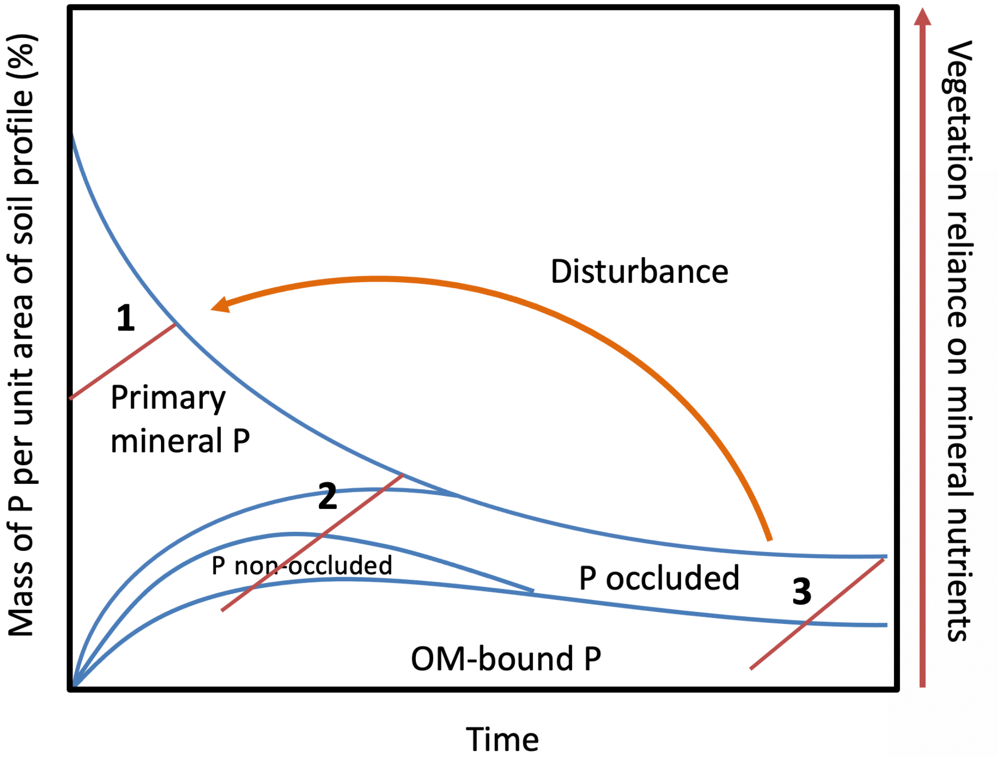

Figure 6. Conceptual diagram reframing Walker and Syers' 1976 hypothesis. Within a given, relatively short, time frame (e.g., regions 1 through 3), as rooting systems develop nutrient economies will develop that are more mineral focused and less OM focused (red lines). The sites in this study are most likely representative of region 2. Both natural and human-induced disturbances that induce secondary succession can reset the system in terms of P forms and rooting system development. As rooting systems develop (moving upslope on red lines), ecosystem reliance on deep, mineral sources of P increases (right Y axis) even as total system P declines over soil development timescales.

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Chapter 2: Global-scale shifts in Anthropocene rooting depths pose unexamined consequences in critical zone functioning

Hauser, E., Sullivan, P.L., Flores, A.N., Hirmas, D., & Billings, S.A. (2021). Global-scale shifts in Anthropocene rooting depths pose unexamined consequences in critical zone functioning. *Earth's Future, in revision*.

Abstract

Rooting depth is an ecosystem trait that determines the extent of soil development and carbon (C) cycling. Recent hypotheses propose that human-induced changes to Earth's biogeochemical cycles propagate deeply due to rooting depth changes from agricultural and climate-induced land cover changes. Yet, the lack of a global-scale quantification of rooting depth responses to human activity limits knowledge of hydrosphere-atmosphere-lithosphere feedbacks in the Anthropocene. Here we use land cover datasets to demonstrate that global rooting depths have become shallower in the Anthropocene and are likely to become yet shallower this century. Specifically, globally averaged depths above which 99% of root biomass occurs (D99) are 17%, or 26 cm, shallower relative to those for potential vegetation, representing a loss of ~37,700 km^3 of rooted volume. This net shallowing results from agricultural expansion truncating D99 by 65 cm, and woody encroachment linked to anthropogenic climate change extending D99 by 41 cm. Projected land cover scenarios in 2100 suggest further D99 shallowing of up to 59 cm, generating losses of rooted volume of ~85,600 km^3, values exceeding root losses experienced to date and suggesting that the pace of root shallowing will quicken in the coming century. Losses of Earth's deepest roots — soil-forming agents — suggest unanticipated changes in fluxes of water, solutes, and C. Two important messages emerge from our analyses: human-modified root distributions should be incorporated into global models, and a significant gap in deep root research, both empirical and theoretical, inhibits accurate projections of future root distributions and their biogeochemical consequences.

Introduction

Roots are subsurface engineers, and their distributions drive ecosystem-scale processes (Maeght et al. 2013; Pierret et al. 2016; Sullivan et al. *accepted*) such as soil development (Brantley et al. 2017; Hasenmueller et al. 2017; Austin et al. 2018), release of mineral-bound nutrients (Jobbagy and Jackson 2001; Hasenmueller et al. 2017; Austin et al. 2018), subsoil water flow paths and residence time (Zhang et al. 2015; Fan et al. 2017) , and deep C fluxes (Richter and Markewitz 1995; Schenk 2007; Pierret et al. 2016; Fan et al. 2017; Billings et al. 2018). The dominant drivers of rooting distributions are plant functional type (PFT, Jackson et al. 1996) and variation in water availability (Schenk 2007; Nippert et al. 2007; Fan et al. 2017), both of which are changing in response to anthropogenic land cover conversion, as well as altered atmospheric composition and concomitant changes in climate (Edgeworth et al. 2001; Cramer et al. 2010; Ellis et al. 2010). This observation suggests that rooting depth distributions have undergone changes due to human activities in the critical zone (CZ, Earth's living skin, Jordan et al. 2001) and will likely continue to do so as anthropogenic forcings proceed.

Quantifying large-scale, human-induced changes to rooting distributions and how they may differ regionally is a critical step towards a greater understanding of how roots govern large-scale, sub-surface and surface processes. In spite of widespread recognition of the importance of root depth (Maeght et al. 2013; Pierret et al. 2016) and a growing recognition of the great depths to which roots can penetrate (Nepstad et al. 1994; Canadell et al. 1996; Schenk and Jackson 2002a; Schenk and Jackson et al. 2002b; Fan et al. 2017), large-scale responses of rooting depths to anthropogenic perturbations of the biosphere have been poorly characterized. This knowledge gap is due in part to the challenges of accessing relatively deep soil horizons (Maeght et al. 2013), as well as the challenge of unraveling the vast complexity of Earth's subsurface systems.

One consequence of poorly defined rooting distributions at large spatial scales is generalized representations of rooting parameters in land models (McCormack et al. 2015; Iversen et al. 2017; McCormack et al. 2017). Although many land models, such as the Community Land Model (CLM), represent changes to roots with land use change (Lawrence et al. 2019), some land cover types are not well represented in these models. For example, crops in CLMs are assigned the same rooting depth as C3 grasses (Lawrence et al. 2019), though row crops in particular typically have far shallower roots than perennial plants (Canadell et al. 1996; DuPont et al. 2014; Billings et al. 2018). Given the plethora of CZ functions influenced by roots (Maeght et al. 2013; Pierret et al. 2016), poor characterization of rooting depths likely limits the accuracy of projected responses of the coupled terrestrial water, energy, and C cycles to climate in the Anthropocene.

Two Anthropocene phenomena occur at sufficient magnitude to alter rooting distributions in ways complicating their quantification. First, many regions have experienced conversion to annual row crops (Ramankutty and Foley 1999; Ellis et al. 2010), a process that induces mortality of deep perennial root systems and replaces them with relatively shallow roots (Billings et al. 2018). In contrast, climate change and increasing atmospheric CO_2 concentrations are linked to root extension of extant woody plants (Iversen 2010) and shifting ecoregion ranges may increase rooting depths where more deeply rooted woody vegetation becomes increasingly abundant in grasslands and tundra (Jackson et al. 1996; Harsch et al. 2009; Stevens et al. 2017; Wang et al. 2019). Studies exploring rooting depth typically focus on absolute rooting depths and their responses to climate or atmospheric CO_2 (Kleidon and Heimann 1998; Kleidon 2003) or, separately, land cover changes in specific regions of interest (Jeremillo et al. 2003; Hertel et al. 2009; DuPont et al. 2010). Despite known changes in global land cover (Ellis et al. 2010) that are associated with distinct rooting depths (Jackson et al. 1996; Zeng 2001), as well as global analyses of the maximum extent of contemporary root depths (Schenk and Jackson 2002a; Schenk and Jackson 2002b; Schenk and Jackson 2005), to date, no one has directly quantified the net change in rooting distributions at the global scale as a consequence of these opposing human activities.

Here, we make a first estimate of the extent to which rooting depths increase or decrease in response to land use and climate change and the volume of soil this change represents. We also project how rooting depths and volumes may change throughout the 21st century as more land is converted to agricultural and urban use, and as biome ranges continue to shift with changing climate. We emphasize that our focus is not on maximum rooting depths. Indeed, there is a growing appreciation of the great depths to which vegetation can root (Schenk and Jackson 2002a; Schenk and Jackson 2005; Maeght et al. 2013; Pierret et al. 2016; Fan et al. 2017) though the true maximum rooting depth may never be known in some systems (Kleidon 2003; Pierret et al. 2016; Fan et al. 2017). Instead, we focus on the depths to which most or half (i.e., 99%, 95%, and 50%) of ecosystems' root biomass extends (Zeng 2001), as well as changes to rooted soil volume. These metrics that highlight very deep roots, the depths at which most roots reside, and the soil volume over which roots are changing, are functionally consequential measures. These metrics represent those for which much data exist, thus facilitating the cross-system comparisons necessary to estimate the spatial extent of rooting depth changes in the Anthropocene. Our work thus reveals how anthropogenic, global-scale changes in rooting depth metrics are changing, thereby illuminating critical next steps to help us understand future CZ functioning.

Materials and Methods

We estimated the volume of soil influenced by human-promoted modification of root distributions. To do this, we estimated potential (i.e., no human influence), contemporary, and projected root distributions at the global scale by combining biome-specific rooting depth functions derived from empirical studies (described below) with spatially explicit land cover datasets. As a part of this process, we examined multiple datasets that, in theory, could help us estimate how humans modify rooting distributions. First, we offer a description of selected datasets followed by an explanation of our selection from those available.

We used satellite-derived, potential vegetation representing 15 land cover classes (Haxeltine and Prentice 1996) and their potential global distribution in the absence of human activity at a 5-minute spatial resolution (Ramankutty and Foley 1999). We compared potential vegetation classes to contemporary land cover as defined by the Global Land Cover 2000 (GLC2000) dataset (Bartolome and Belward 2005). GLC2000 represents 22 land cover types, which are designated according to plant functional types ascribed to satellite images and ground-truthed by regional analysts. We aligned contemporary vegetation classifications with potential vegetation classes according to previously published frameworks for ecoregion designation (Bartolome and Belward 2005) and augmented these classes to include a class for permafrost regions where rooting depth is likely limited (Billings et al. 1997; Boike et al. 2018). These efforts resulted in 25 distinct land cover types for which rooting depths were assigned. Projected vegetation classes were similarly developed for four Shared Socioeconomic Pathway (SSP) and Representative Concentrations Pathway (RCP) scenarios using spatial projections of gridded, 0.5° x 0.5° resolution land covers for the year 2100 (Hurtt et al., 2011).

For all vegetation datasets except those above 60°N latitude (described below), we estimated biome-specific rooting depths by assigning rooting depth functions derived from empirical data compiled in the Fine Root Ecology Database (FRED) and the National Ecological Observatory Network (NEON) database (Iversen et al. 2021; NEON 2021). Specifically, we estimated the depths by which rooting systems exhibit 50% (D50), 95% (D95) and 99% (D99) of their total biomass in each land cover type. To generate rooting depth functions, we assigned FRED and NEON rooting depth data to biomes according to each datapoint's position on our modified GLC2000 land cover map. Each set of points was checked using Google Earth to ensure that datapoints were correctly assigned. Due to the resolution of the GLC2000 map, some shrubland and woodland categories were incorrectly identified as cropland; for these points, we reassigned shrub-covered areas to the open-closed deciduous shrubland class and woodlands to the open broadleaved deciduous forest class. We then bootstrapped fitted decay curves to each set of points for each biome using the model presented by Zeng (2001). Invoking these functions (Zeng 2001) assumes that rooting depth distributions remain similar for each vegetation functional type in the potential, contemporary, and future scenarios. The merit of this assumption may vary with time but keeping each biome's rooting depth consistent across the Holocene and into the future allows us to parse the influence of land cover change on rooting depths from that of less well-characterized phenomena.

Because it was challenging to match the land cover classifications used in vegetation maps to biome classifications for which we have rooting depth equations, we modified the estimated rooting depth distributions for four of the 25 land covers. First, the land cover datasets combine both polar and mid-latitude deserts into a single desert category based on hydrologic regimes, yet rooting depths in polar deserts are often constrained by permafrost. We thus separated these two desert regions, reassigning deserts in polar regions to the ‘tundra’ classification above 60°N (Zhang et al. 2008). Second, we reassigned evergreen forest and mixed vegetation classes above 50°N to the ‘boreal’ vegetation classification (Brandt et al. 2013; Price et al. 2013), and ecoregions above 60°N to the class ‘tundra’ (Zhang et al. 2008) for maps of land cover. For maps of rooting depth, we gave all classes above 60°N a rooting depth specific to permafrost-underlain regions, where roots typically do not penetrate deeper than 30 cm (Billings et al. 1997; Zhang et al. 2008; Boike et al. 2018). Finally, because many remote sensing-based studies omit large, lower latitude desert regions from their analyses due to the lack of quantifiable ecosystem productivity in these systems (Zhao et al. 2005), we omitted mid-latitude deserts from rooting depth averages reported in the main text. Instead, we present rooting depth metrics that incorporate these mid-latitude deserts’ potential contribution to global root averages in Table 1 of the Supporting Information. Comparison of these results with those reported in the text reveal an inflated influence of mid-latitude desert rooting depth estimates on global averages that likely does not represent reality due to the low density of plants in true deserts (Whitford and Duval 2019).

To assess potential effects of global-scale perturbations projected by the year 2100 on rooting depth distributions, we examined multiple SSP and RCP land cover projections from the Intergovernmental Panel on Climate Change (IPCC). Projected vegetation classes were developed for 4 SSP RCP scenarios (SSP2 RCP4.5, SSP1 RCP2.6, SSP4 RCP6.0, SSP5 RCP8.5). Landuse Harmonization datasets designate land cover classes more coarsely than either GLC2000 or potential vegetation datasets, delineating primary and secondary forest regions, primary and secondary non-forest regions, five agricultural classes, pastureland, rangeland, and urban regions (Hurtt et al. 2011). We assigned a rooting depth equation derived from agricultural croplands in the FRED and NEON datasets to all 5 agricultural classes in the Landuse Harmonization dataset. For secondary non-forests, pastures, and rangelands we assigned rooting depth equations representing herbaceous and grassland systems in the FRED and NEON datasets. Because most secondary forests in these scenarios were in the boreal region, we assigned the average root depth value of mixed forests (130 cm) and boreal forests (85 cm) to secondary forests. Reflecting anticipated warming, rooting depths assigned in all future scenarios removed permafrost constraints (Lawrence and Slater 2005).

We examined multiple datasets describing contemporary global root distributions (Schenk and Jackson 2009) and landcover scenarios across time (Hurtt et al. 2011) as potential candidates for addressing the degree to which humans modify the rooted volume of Earth's subsurface. Such datasets have been pivotal in developing our understanding of and appreciation for the depths of deep roots (Schenk and Jackson 2005; Schenk 2005; Pierret et al. 2016), and the Landuse Harmonization (LUH) scenarios represent the best available data for future land cover classifications to date (Hurtt et al. 2020). However, the Schenk and Jackson dataset does not describe roots in agricultural lands, ploughed and fertilized lands, or wetlands (Schenk and Jackson, 2005), and is not divided into land cover classes that can be integrated with datasets describing potential and future land cover scenarios. The LUH scenarios combine land cover classes in ways that result in the loss of important nuances in root distribution estimates in past and contemporary scenarios. For example, all forest types in LUH scenarios are grouped into 'secondary' and 'primary' forest rather than more region-specific forest classifications (Hurtt et al. 2020). In contrast, employing the GLC2000 vegetation classes with rooting depths derived from FRED and NEON data, which include Jackson et al.'s (1996) data, permitted us to examine two key features of interest. First, this approach permitted incorporation of agricultural land cover classes, a feature that is absent in datasets featuring root distributions alone. Second, the Ramankutty and Foley (1999) dataset serves as the only spatially quantified representation of the potential land cover in the absence of human activity at a 5-minute resolution, allowing for detailed backcasting of estimates of human-induced changes to roots.

Using R's raster package (RStudio Team 2017; Hijmans et al. 2019) we assigned rooting depth values to each land cover classification of the potential, contemporary, and projected vegetation maps, and calculated global means of each depth metric. After determining the differences in rooting depths across scenarios, we examined the spatial extent of depth changes to determine differences in rooted volume across scenarios. We then compared metrics across time using 95% confidence intervals of the mean estimates of global rooting depth metrics. Deep root estimates, particularly D99, reflect meaningful uncertainty (Schenk and Jackson 2002b). However, the relative means of biomes' rooting depth metrics permit assessment of the combined influence of changes in those metrics with biomes' spatial extents on average, global root depth distributions. We performed correlated t-tests on pairs of rasters (i.e., potential vs. contemporary, and contemporary vs. projected) to determine whether differences between these estimated rooting depth metrics are significantly different from zero. Data were assessed to ensure they met the assumptions of correlated t-tests, including independence of observations, normal distribution of the dependent variable, and no dependent variable outliers. Where data did not meet the assumptions, we ran Wilcoxon tests on the dataset pairs to assess differences in root depth metrics and reported the V-statistics and p values generated from those tests.

Results

Comparisons of potential and contemporary land cover (Figures 1a and b) and their estimated rooting depths (Figures 1c and d) suggest that spatially averaged, global values of D99 are up to 17% shallower (~26 cm) under contemporary land cover distributions than if potential vegetation cover types covered Earth's terrestrial surface (V = 5.3847e+11, Wilcoxon *P* < 0.0001; Figures 1c and d, Table S1). This represents a loss of rooted volume of ~37,700 km^3. Values of D95 for contemporary land cover also express trends of root shallowing, though less so than D99 (17% or 15 cm, loss of ~21,800 km^3; V = 5.4244e+11, Wilcoxon *P* < 0.0001; Figures S1a and b). Depth to 50% root biomass (D50), by comparison, displays relatively less variation between contemporary and potential land cover, becoming ~1 cm shallower (14%, ~1450 km^3 ; V = 7.4318e+11, Wilcoxon *P* < 0.0001) on average (Figure S2). The comparatively small change in globally averaged D50 values is a consequence of relatively rapid shallow root establishment of cultivated systems.

Agricultural land conversion serves as the dominant influence on these global trends (Figures 2 and 3). Where perennial vegetation has been converted to agricultural land (defined here as annual crops and managed pasture), D99 has decreased by as much as 35% (65 cm) across 2.4 x 10^9 ha (15% of Earth's terrestrial surface). Where woody encroachment is evident in contemporary land cover data, D99 increased relative to potential vegetation by up to 28% (41 cm; note that here we use the phrase 'woody encroachment' to refer to both shrubland encroachment into grasslands, and forest encroachment into Arctic and alpine tundra). This result may overestimate current rooting depths if the rooting depths we assigned were derived from well-established, mature systems, given that woody plants in recently encroached systems likely have not yet achieved such depths (Stevens et al. 2017; Billings et al. 2018). In spite of this possible overestimation, root deepening via woody encroachment does not overcome the effect of root shallowing in agricultural lands, in part because of the smaller fraction of Earth's terrestrial surface experiencing woody encroachment (9.3 x 10^7 ha, or 0.6%).

Changes to rooting distributions by the year 2100 vary under different potential scenarios of climate and land use change as well as different societal responses to those changes. The SSP scenarios examined here represent global narratives including a scenario with few roadblocks to both mitigation of and adaptation to climate change (SSP1), moderate challenges to mitigation and adaptation (SSP2), a scenario of social inequality with many challenges to adaptation but few for mitigation (SSP4), and a strategy of fossil fuel dependence with many challenges to mitigation but few to social adaptation (SSP 5, Riahi et al. 2017). These narratives are used in conjunction with projected land use and climate (RCP) scenarios to model future societal and ecological conditions, which we rely on for our rooting distribution estimates.

Projections for the year 2100 suggest that the scenario with the largest cropland increase and relatively low radiative forcing enhancement from current levels (SSP1 RCP2.6, Figure 4a) generates the most extreme reduction of deep roots, truncating values of D99 by 59 cm ($V = 2.8106e+10$, Wilcoxon $P < 0.0001$). The smallest decline in D99, a shallowing of 53 cm ($V = 2.7448e+10$, Wilcoxon $P < 0.0001$), occurs under a scenario of moderate cropland increase and stabilization of moderate to high radiative forcing at 6 Wm^{-2} by 2100 (SSP4 RCP6.0, Figure 4b). As a result, the future rooted volume will be reduced by ~76,900 km^3 to ~85,600 km^3. The highest emissions scenario (SSP5 RCP8.5) produces an intermediate D99 shallowing of 55 cm (~79,800 km^3), the result of extensive conversion of forests into cropland (Figure S4) and root elongation in boreal and high-elevation regions.

Values of D50 for the year 2100 also reflect a consistent response to the rapidly transitioning vegetation that likely drives projected changes in D99 and D95, leading to a D50 shallowing of 5 to 6 cm across all assessed scenarios ($V = 2.9228e+10$, Wilcoxon $P < 0.0001$; Figure S5), a soil volume of ~7500 km^3. Though small relative to changes in deep root systems, this D50 shallowing is 4 to 5 cm more severe than that occurring during the previous ~10,000 y (Gupta, 2004) of anthropogenic land conversion to agriculture (Figure S6).

Discussion

Our rooting depth and volume estimates suggest that the portion of rooting biomass most vulnerable to human influence is, counterintuitively, deep in the soil profile (Figures 2 and 3). Although maximum rooting depths are poorly characterized and are likely deeper than is typically appreciated (Maeght et al. 2013; Pierret et al. 2016; Fan et al. 2017), we demonstrate that metrics of most or half of all rooting biomass (i.e., D99, D95, and D50), no matter their absolute value, are currently a reflection of human-induced, global-scale changes in land cover (Figure 1). We further demonstrate that the globally-averaged estimate of a 26 cm shallowing of D99 values and loss of ~37,700 km^3 rooted volume is the net result of root shallowing in agricultural regions and root elongation in regions of woody encroachment, with the area represented by agriculture dominating the effect.

With atmospheric CO_2 anticipated to continue increasing in the coming decades, a factor often implicated as promoting woody encroachment (Devine et al. 2017), we might expect woody encroachment's elongating effects on D99, D95, D50 and rooted volume to effectively mitigate the root shallowing effect of land conversion to agriculture in the future. However, the four IPCC scenarios explored here suggest that by 2100, rooting distributions may become yet shallower relative to contemporary rooting depths (Figures 4, S4 and S5). As observed for comparisons between potential and contemporary land cover, the deeper rooting metrics (D99 and D95) display greater changes in their global mean than D50 when comparing contemporary and projected land cover. Thus, both comparisons suggest that the deepest roots are the most vulnerable to loss via anthropogenic changes.

Reduced rooting depths by 2100 are driven in part by substantial root shallowing across the Amazon basin (Fig. 4), consistent with multiple projections of rapidly transitioning vegetation cover in that region (Hurtt et al. 2011). Future roots also appear truncated in some boreal regions (Fig. 4), contradicting findings of contemporary scenarios in which roots grow deeper in some northern regions. Future dynamics of roots in northern regions are highly uncertain. While some studies hint that roots may deepen as soils thaw (Harsch et al. 2009; Wang et al. 2020), others demonstrate that long term changes in snowpack will produce extremes in soil freeze/thaw cycles that will reduce vegetation survival and rooting depth (Groffman et al. 2001; Blume-Werry et al. 2016). In our future rooting depth scenarios, the 30 cm root depth we applied to permafrost regions was removed to account for the extreme loss of permafrost predicted by the year 2100 (Lawrence and Slater 2005); in spite of the potentially greater rooting depths resulting from permafrost loss, shallower roots were projected in these regions, perhaps reflecting projected regional increases in disturbances (Hurtt et al. 2020). Clarifying the net result of climate and land use change on deep roots in these regions will be critical for understanding future global C and water dynamics.

Unlike contemporary vs. potential vegetation comparisons, D50 metrics in future scenarios are also considerably shallower than contemporary scenarios and represent a greater loss of soil volume. These results highlight that anthropogenically-induced changes in surficial soil horizons' root abundances in the coming decades will likely exceed those of the past several millennia. They also emphasize that even relatively shallow soil horizons (*i.e.*, those expressed by D50), where both natural and agricultural species root, will undergo redistribution in the coming decades.

There are myriad feasible consequences of altered rooting distributions for biogeochemical and hydrological fluxes that prompt intriguing hypotheses. For example, roots beneath the zone of maximum rooting density are attributed with developing the soils that mantle Earth's surface, so much so that they are referred to as the planet's biotic weathering front, where life – roots and microbes – promotes the dissolution of bedrock (Richter and Markewitz 1995; Berner et al. 2003; Brantley et al. 2012; Pawlik 2013; Dontsova et al. 2020). Results from the current study suggest that these biotic weathering forces in many regions do not reach as deeply into the regolith as they did prior to human influence, prompting the hypothesis that the intensity of biotic modes of soil formation at the bottom of the soil profile have declined in the Anthropocene. Further, a smaller volume of soil explored by rooting systems prompts the hypothesis that soil water storage capacity, nutrient replenishment and solute losses from freshly weathered material have declined (Swank 1986; Nepstad et al. 1994; Berner 1998).

Our findings serve as a useful starting point for formulating and probing these hypotheses. The uncertainty embedded in the projections reported here highlights the substantial need for better quantification of rooting distributions in diverse biomes, particularly for deep roots, and how we quantify their future dynamics. Although this study makes a first attempt at measuring the extent of anthropogenically-induced changes in rooting systems, it also points to key knowledge gaps. Foremost, our results reflect a lack of information regarding roots in northern regions both now and in the future (see especially Figure 1c). Additionally, there is a great deal of uncertainty in rooting depth estimates, especially for the deepest roots (Shenk and Jackson 2002). Indeed, many of the deepest roots have been observed incidentally, suggesting that we have not yet sampled roots to their fullest extent (Fan et al. 2017). We suggest that CZ research combining empirical and modeling approaches could help focus future research efforts on these critical gaps.

More empirical studies quantifying the depth distributions of roots are needed to clarify the ways in which global rooting distributions are changing. Specifically, leveraging of on-going climate experiments (e.g., Caplan et al. 2019), naturally existing climatic gradients (e.g., Ziegler et al. 2017), and chronosequences (e.g., Billings et al. 2018) could demonstrate how rooting depths respond to global changes to temperature and precipitation, as well as reveal quantitative relationships between rooting depth distributions and their impacts on soil formation processes, especially at depth. Focusing these studies in regions with relatively less research, such as the high latitude regions highlighted here, will improve our understanding of root-induced processes at the global scale.

Additionally, empirical and modeling studies examining the biogeochemical consequences of rooting depth change are critical. More extensive work either directly measuring subsurface biogeochemical fluxes as they respond to changes in rooting depth distributions, or biogeochemical process modeling that projects such fluxes, will be invaluable for generating input parameters representing subsurface biogeochemical fluxes in ESMs. Because terrestrial vegetation exerts a fundamental global control on land-atmosphere exchanges of water, energy, C, and other elements, improved representation of rooting distributions in global land models such as the Community Land Model (Lawrence et al. 2019) is of critical importance. This is particularly true as more sophisticated aboveground and belowground vegetation and biogeochemical processes are incorporated into these models (e.g., Tang et al. 2013; Fisher et al. 2017; Kennedy et al. 2019). With improved fidelity to biophysical and biogeochemical processes comes the corresponding opportunity to explore the potential consequences of changes in global rooting depths on land-atmosphere exchanges of water, energy, and C, and the large-scale ramifications that changes in rooting depths have for climate. Well-designed numerical experiments could elucidate the relative impacts of exogenous (e.g., agricultural conversion, woody encroachment) versus endogenous (e.g., water and nutrient limitation) drivers of changes in rooting depths on terrestrial cycling of water, energy, and C. These modeling efforts can feedback into empirical studies by illuminating regions where rooting depth knowledge is not sufficient and pointing toward parameters requiring more explicit definition to improve future predictions. Such integrative studies would strengthen the nascent interactions between ESM and CZ communities to address pressing questions about global change that cannot be solved without substantial input from both disciplines (National Academy of Sciences, Engineering and Medicine 2020). The improved representation of changing rooting depth distributions can link these research communities, representing a critical collaboration for understanding current and future functioning of Earth’s critical zone and climate.

Conclusion

Losses of relatively deep roots suggest an overlooked and subtle mechanism by which humans alter soil and ecosystem development. It is well established that humans accelerate losses of surface soil via erosion, which can result in a thinning of Earth’s skin of soil (Wilkinson and McElroy 2007). In contrast, altered rooting depths deep in soil profiles as well as rooted volume due to anthropogenic land use and climate change suggest a means by which human actions may govern soil thickness near the bottom of soil profiles. These shifts in rooting distributions support the idea that signals of the Anthropocene penetrate deeply into the subsurface even in naturally occurring elemental cycles (Billings et al. 2018). Indications of widespread human transformation of land cover across millennia (Edgeworth et al. 2015) imply that reductions in deep root abundances have been underway in multiple regions for a similarly lengthy time. Though improving process representation in land models continues apace (Fisher and Koven 2020), the representation of rooting depth distributions remains largely a static function of only PFT (cf. Drewniak 2019). We present an opportunity to advance the representation of roots in land models by better constraining how rooting depth distributions vary with global change, as well as by identifying specific ecological processes particularly suited to better quantifying the dynamics of rooting, both past and future (e.g., regions of woody encroachment). Co-designed modeling, field and lab studies are needed to help clarify the consequences of rooting depth changes for contemporary and future CZ development. Such studies can elucidate the ways that surficial anthropogenic activities radiate deep within Earth's subsurface, altering the developmental pace and character of Earth's CZ.

Data Availability and Code Availability

The original GLC2000 dataset modified for this analysis can be accessed at https://forobs.jrc.ec.europa.eu/products/glc2000/products.php. The unmodified potential vegetation data can be found at https://daac.ornl.gov/cgi-bin/dsviewer.pl?ds_id=961. All future land use projections can be accessed through the Landuse Harmonization data portal at http://luh.umd.edu/data.shtml. Rasters modified as described in Methods for contemporary and potential land cover, along with root depth assignment .csv files and code are available on Zenodo (https://doi.org/10.5281/zenodo.4298997).andpotential land cover, along with root depth assignment .csv files and code are available on Zenodo (https://doi.org/10.5281/zenodo.4298997).

Figures

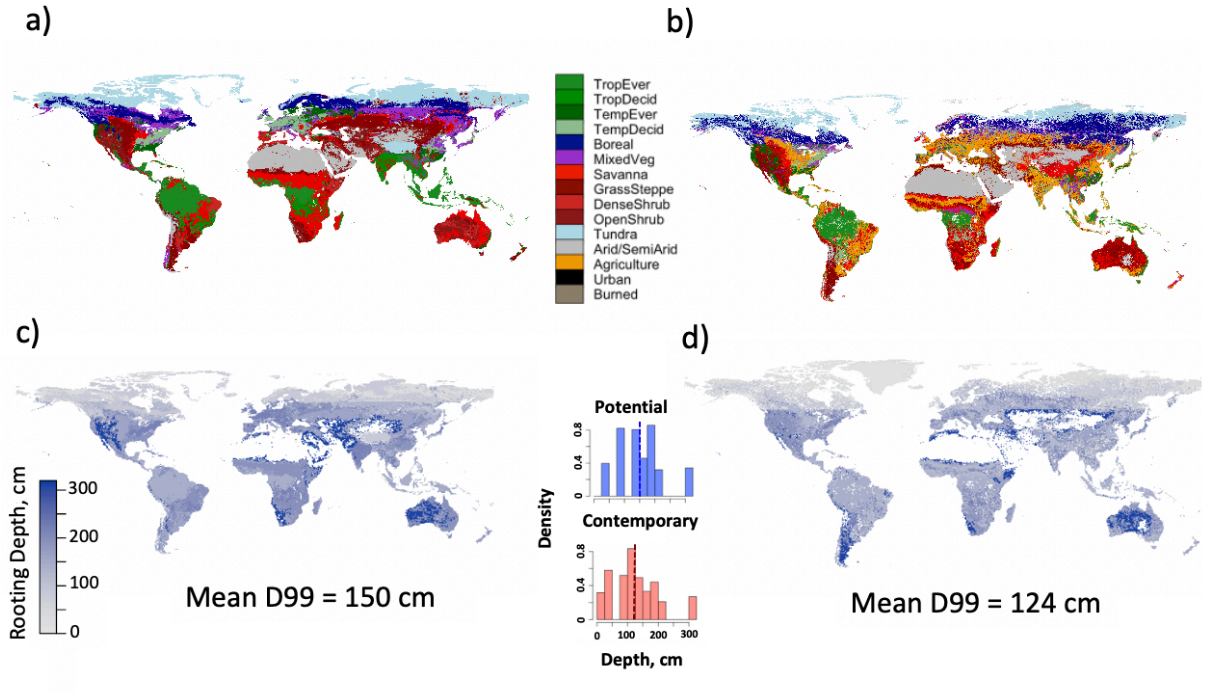

Figure 1. Land cover and associated rooting depths under potential vegetation in the absence of human influence (left column) and today's vegetation distribution (right column). (a) Potential vegetation cover in the absence of human activity modified to accommodate permafrost regions, where all plants regardless of functional type are depth-limited by frozen soils. (b) Contemporary land cover distribution from Global Land Cover 2000 (GLC2000), modified to correspond to potential vegetation land cover classifications. Subsequent maps depict depths by which 99% of rooting biomass occurs (D99) under potential (c) and contemporary (d) land cover types. Inset histogram displays rooting depth distributions. Blue histogram reflects potential vegetation data, and red histogram contemporary land cover. Dashed vertical lines represent means. Appearance of a distinct line at 60°N in potential D99 values in (c) is an artifact of restricted maximum rooting depth assignments to reflect limitations imposed by frozen soils; this distinction is less evident in contemporary D99 maps because of the higher spatial resolution of the GLC2000 dataset. Appearance of a distinct line at 50°N, especially evident in (d), reflects reassignment of mixed forests to the boreal forest class above this latitude (Brandt et al. 2013; Price et al. 2013). While these lines are unrealistic, it reflects our current knowledge about root depths in northern regions and demonstrates the remaining need for additional work combining cryospheric studies and soil science to characterize root systems near polar regions. Note that most of Greenland is assigned a rooting depth of zero in all maps because of ice cover, which is denoted in white and grey in potential and contemporary root coverages, respectively.

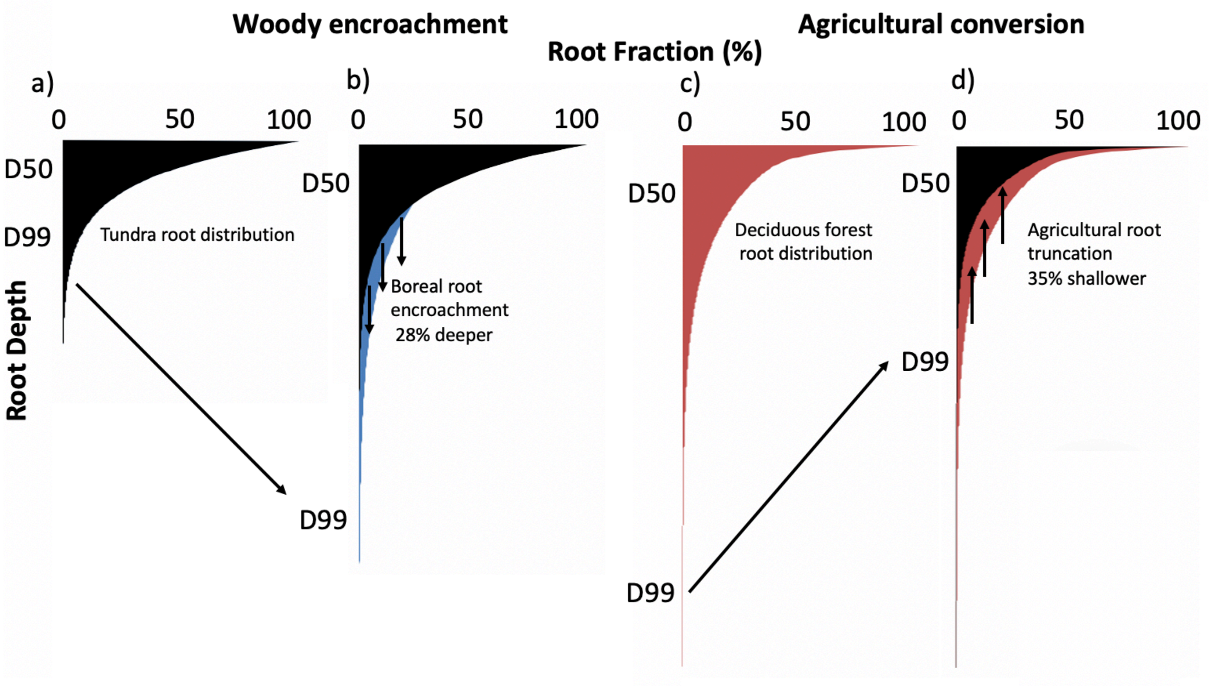

Figure 2. Representation of rooting depth elongation due to woody encroachment (a and b) and rooting depth truncation due to agricultural expansion (d and d). Blue region in B demonstrates the belowground increase in roots shown in blue in Figure 3. Red region in D exemplifies loss of rooting system depth for red regions in Figure 3.

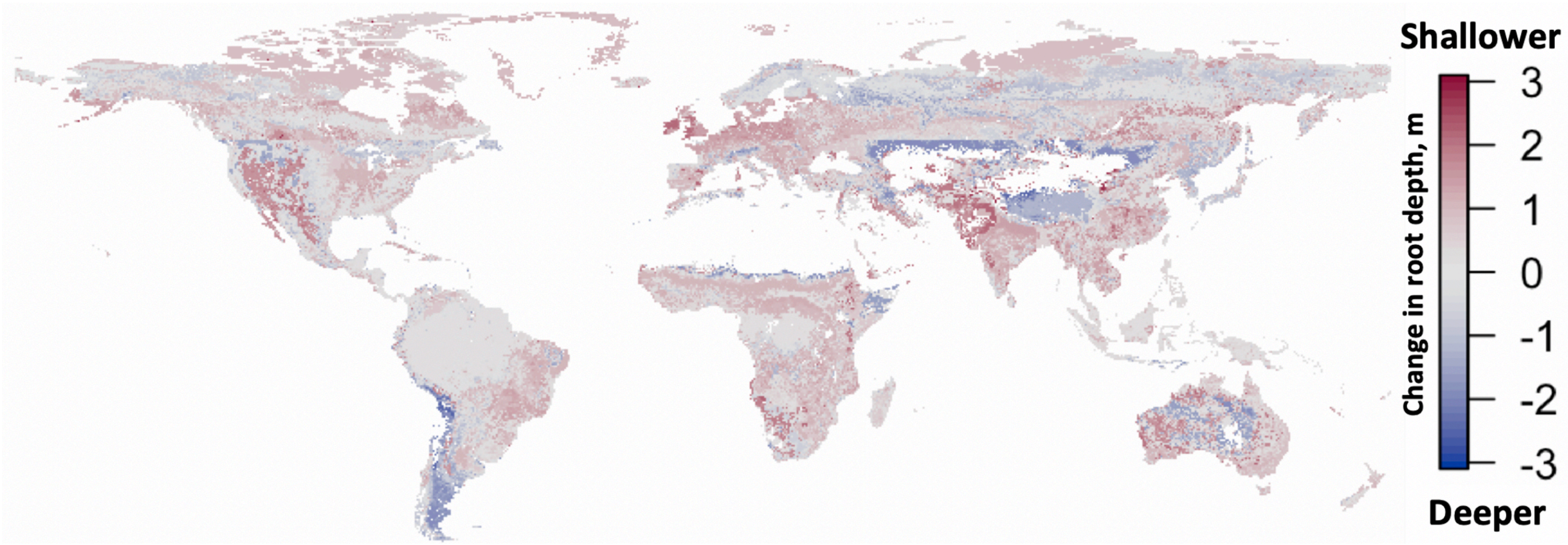

Figure 3. Mapped differences between potential and contemporary rooting depths. Red cells indicate a decrease in the depth to 99% of rooting biomass (D99) while blue cells indicate an increase in D99 resulting from contemporary vegetation distributions. Appearance of a distinct color change at 50°N reflects reassignment of mixed forests to the boreal forest class above this latitude (Brandt et al. 2013; Price et al. 2013).

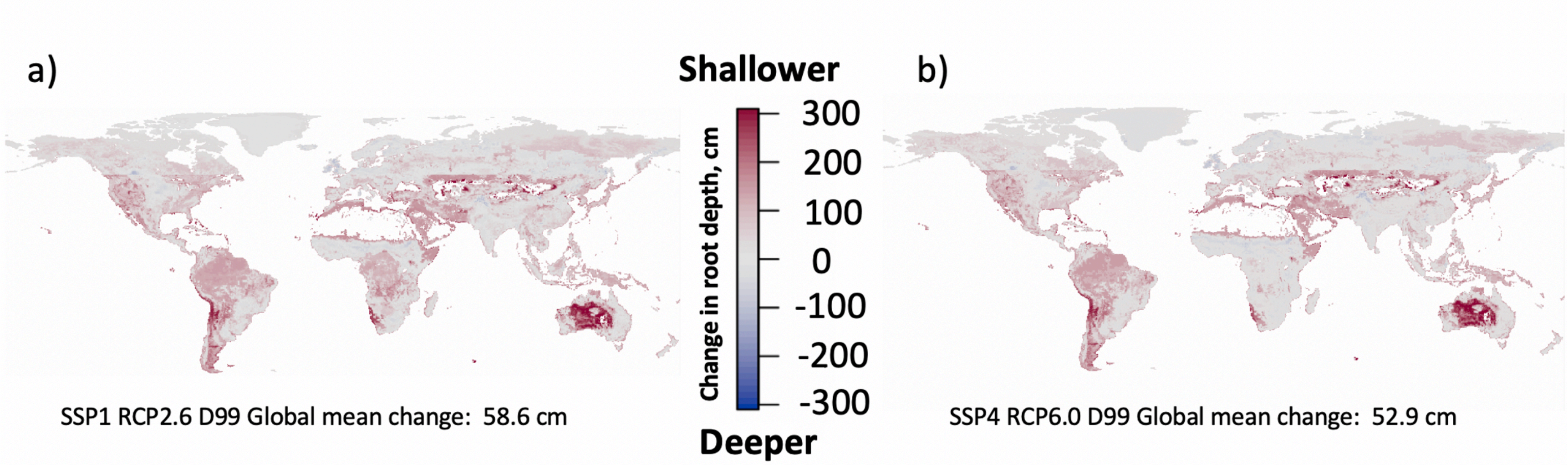

Figure 4. Projected changes of depth to 99% rooting biomass (D99) by the year 2100 relative to contemporary rooting depth distributions. Projections are based on land use and emissions changes under two combinations of Shared Socioeconomic Pathways (SSP) and Representative Concentration Pathways (RCP), SSP1 RCP2.6 (a) and SSP4 RCP6.0 (b). These two maps represent the scenario of greatest projected change and least projected change. Red colors indicate root depth truncation and blue indicates elongation. Appearance of a distinct color change at 50°N reflects reassignment of mixed forests to the boreal forest class above this latitude (Brandt et al. 2013; Price et al. 2013).

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Chapter 3: Persistent anthropogenic legacies structure the depth dependence of regenerating rooting systems and their functions

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Abstract

Biotically-mediated weathering helps to shape Earth's surface. For example, plants expend carbon (C) to mobilize nutrients in forms whose relative abundances vary with depth. It thus is likely that trees' nutrient acquisition strategies – their investment in rooting systems and exudates – may function differently following disturbance-induced changes in depth of rooting zones and soil nutrient stocks. These changes may persist across centuries. We test the hypothesis that plant C allocation for nutrient acquisition is depth dependent as a function of rooting system development and relative abundances of organic vs. mineral nutrient stocks. We further posit that patterns of belowground C allocation to nutrient acquisition reveal anthropogenic signatures through many decades of forest regeneration. To test this idea, we examined fine root abundances and rooting system C in organic acid exudates and exo-enzymes in tandem with depth distributions of organically- and mineral-bound P stocks. Our design permitted us to estimate C tradeoffs between organic vs. mineral nutrient benefits in paired forests with many similar aboveground traits but different ages: post-agricultural mixed-pine forests and older reference hardwoods. Fine roots were more abundant throughout the upper 2 m in reference forest soils than in regenerating stands. Rooting systems in all forests exhibited depth-dependent C allocations to nutrient acquisition reflecting relative abundances of organic vs. mineral bound P stocks. Further, organic vs. mineral stocks underwent redistribution with historic land use, producing distinct ecosystem nutritional economies. In reference forests,

rooting systems are allocating C to relatively deep fine roots and low-C exudation strategies that can increase mobility of mineral-bound P stocks. Regenerating forests exhibit relatively shallower fine root distributions and more diverse exudation strategies reflecting more variable nutrient stocks. We observed these disparities in rooting systems' depth and nutritional mechanisms even though the regenerating forests have attained aboveground biomass stocks similar to those in reference hardwood forests. These distinctions offer plausible belowground mechanisms for observations of continued C sink strength in relatively old forests, and have implications for soil C fates and soil development on timescales relevant to human lifetimes. As such, depth-dependent nutrient returns on plant C investments represent a subtle but consequential signal of the Anthropocene.

Introduction

There is growing appreciation of the diverse roles that tree roots play as soil architects even deep within soil profiles (Richter et al. 2007; Roering et al. 2010; Brantley et al. 2017) and as mediators of unexpectedly large material and energy fluxes at the ecosystem scale (Yin et al. 2014; Finzi et al. 2015; Mayer et al. 2018). Tree roots influence hydrologic flow paths (Bond et al. 2008; Band et al. 2014; Fan et al. 2017), mine soil profiles for nutrients from soil minerals (Lucas 2001; Jobbagy and Jackson 2004; Soper et al. 2018) and organic matter, (Adams and Pate 1992; van Vuuren et al. 1996; Aber and Melillo 2001; Janzen 2006; Newmann 2007), can both slow and accelerate erosion (Brantley et al. 2012; Pawlik 2013), and redistribute nutrients in ways that influence metrics of mineralogy (Austin et al. 2018) and soil development (Brantley et al. 2012; Brantley et al. 2017). They also are responsible for pumping fixed carbon (C) into the subsoil via root-respired CO_2 (Nadelhoffer and Raich 1992; Lambers et al. 2002; Ryan and Law

2005; Billings et al. 2018; Cherkinsky et al. 2018), the CO_2 respired by microbes dependent on them (Scheckenberger et al. 2008; Keiluweit et al. 2015), root and microbial C exudates (Grayston et al. 1996; Lynch and Ho 2005; Thornley et al. 2015), and the construction of roots themselves (Nadelhoffer and Raich 1992; Jackson et al. 1997). Although years of research highlight the millennia over which roots have been helping to construct Earth's skin (Berner 1992; Kelly et al. 1998; Berner et al. 2003), deep soil processes are a historically understudied trait (Richter and Billings 2015) and deep roots likely have a disproportionately strong influence over ecosystem functions (Maeght et al 2013, Pierret et al. 2016). Indeed, deep root activities have recently earned trees the designation of "builders and plumbers of Earth's Critical Zone" (Brantley et al. 2017).

Human-driven alterations in aboveground biotic communities can result in persistently altered biogeochemical signals beneath the surface (Ellis 2011; Walters et al. 2016). We focus on two specific, common phenomena relevant to root behavior. First, deeply rooted, long-lived plants have been replaced with more shallowly rooted ecosystems in many regions, especially where annual, row-crop agriculture is a dominant land use (Canadell et al. 1996; Fan et al. 2016). However, where agricultural lands are abandoned, regenerating forests are common; this is especially true in the northern hemisphere (World Bank 2015). Despite aboveground shifts toward earlier successional species, regenerating systems can still exhibit many of the qualities of older forests with minimal human imprint (Eaton and Lawrence 2006; Martin et al. 2013; Billings et al. 2018); for example, relatively young forests can achieve similar hydrologic functioning as much older forests after just 20 years (Jipp et al. 1998). However, root networks require many decades or centuries to establish structures comparable in depth and complexity to much older forests (Dupouey et al. 2002; Knops and Bradley 2009; Devine *et al.* 2011; Yuan &

Chen, 2012; Pierret 2016; Rodina et al. 2019), and deep root networks can be important promoters of long-term soil development (Jobaggy and Jackson 2004; Brantley et al. 2012). For example, even after ~80 y of forest regeneration rooting depth distributions lag those of older reference forests, with consequences for biogeochemical fluxes far below the zone of maximum rooting density (Billings et al. 2018; Cherkinsky et al. 2018). Although some of these changes may be a consequence of species changes across successional stages, the shift to an earlier successional stage is itself a consequence of land use change. Thus, a relative dearth of deep roots, whether due to species or ecosystem age, can serve as an important signal of the Anthropocene in many subsoils.

Second, regenerating rooting systems grow where anthropogenically accelerated erosion (Haff 2010; Brecheisen et al. 2019) and historic mineral fertilizer use (Richter and Markewitz 2001; Richter et al. 2006; Yoo et al. 2015) have altered the geomorphology of Earth's surface and the distribution of mineral nutrients across it, respectively. Compared to unmodified soil profiles, contemporary soils can exhibit proportionately more mineral-bound nutrients near the surface due to historically accelerated erosion of topsoil typically enriched in organically-bound nutrients (Richter and Markewitz 2001; Yoo et al. 2015), as well as past application of mineral fertilizers (Richter et al. 2006). Redistribution of the depths and relative abundances of mineral vs. organic nutrients in disturbed ecosystems compared to what is observed in relatively undisturbed ecosystems represents another way in which anthropogenic signals emerge in Earth's subsurface where the aboveground system may otherwise appear natural.

Combined, these widespread phenomena – shallower roots where ecosystems have been disturbed frequently and a greater relative abundance of mineral-bound nutrients in shallower horizons – suggest that trees' means of obtaining nutrients may be different where historic, agricultural land use has been extensive. Rooting system nutritional strategies are complex, encompassing both root proliferation and distinct mechanisms to access organic vs. mineral nutrient forms. Here, we use “rooting system” to acknowledge that roots and the microbes that reside in and around them jointly exercise these nutrient acquisition strategies. One such strategy is organic anion production, which enhances mineral-bound nutrient mobility via desorption, ligand exchange, and pH-based reactions (Landeweert et al. 2001; Marschner and Rengel 2007). Rooting systems also can exude extracellular enzymes that hydrolyze organic molecules, transforming nutrients bound in relatively large organic compounds into bioavailable forms (Marschner and Rengel 2007). These two mechanisms incur distinct costs of fixed C. Extracellular enzymes exhibit nearly double the concentration of C as organic acids (Smith 1976; Hauser and Billings, unpublished data), suggesting that release of bioavailable nutrients via extracellular enzymes may require relatively greater C investment. However, the relatively shallow rooting systems of young, regenerating forests may be restricted to more shallow nutrient pools, which tend to accumulate organic stocks as forests regrow (Cross and Schlesinger 1995; Vitousek et al. 1997; Mobley et al. 2013). In contrast, more deeply rooted, older forests may have greater access to deeper nutrient sources that are dominantly mineral-bound (Jin et al. 2010; Brantley 2012; Hasenmueller et al. 2017) and may be subject to redistribution via nutrient uplift (Jobaggy and Jackson 2001).

One nutrient element for which these acquisition strategies are especially evident is phosphorus (P), which we use as an element of interest in this study due to its presence in both organic- and mineral-derived sources, and its status as a macronutrient in high demand across Earth’s ecosystems (Vitousek et al. 1997). Rooting systems display the mechanisms described above as a means of obtaining P. For example, phosphatase enzyme exudation of rooting systems occurs in the presence of organic P (Bunemann et al. 2011). Additionally, production of organic acids such as oxalic acid, though not specific to P acquisition, can manipulate soil pH and increase mineral-bound P mobilization (Marschner and Rengel 2007; Thorley et al. 2015). Numerous studies also suggest that root and mycorrhizal morphology reflects P bioavailability. Proliferation of high surface area fine roots, root hairs, and mycorrhizal mycelia represent key plant mechanisms for tapping into heterogeneous soil P supplies (Hodge et al. 2004; Liu et al. 2015; Lugli et al. 2019) that preferentially occur near concentrated P sources (Drew 1975; Jackson et al. 1990). Plant P acquisition thus represents a considerable investment of C because it requires multiple mechanisms including both root and mycorrhizal proliferation and exudate production.

Because of these varying C costs for obtaining different P forms and the unpredictable belowground conditions regenerating ecosystems can experience, we might expect ecosystems regenerating on lands that experienced intense land use in the past to exhibit different P acquisition mechanisms relative to less disturbed systems. These strategies should reflect efficient tradeoffs between C costs and the potential nutrient benefits offered by varying depth distributions of organic vs. mineral nutrient stocks. We thus hypothesize that plant C allocation to mechanisms of nutrient acquisition is strategically depth dependent as a function of 1) degree of rooting system development and 2) the relative abundance of mineral vs. organic P forms in the rooting zone. Further, we posit that, because land use history influences rooting system depth development as well as stocks and depths of nutrient sources, forest C allocation to specific nutrient acquisition mechanisms reveals anthropogenic signatures lasting for many decades after initiation of forest regrowth. If validated, these hypotheses suggest that the functional signatures of land use legacies dictate contemporary nutrient economies that serve as underappreciated drivers of soil organic C (SOC) distributions. Thus, forest disturbance and subsequent regrowth may prompt shifts in deep critical zone phenomena that drive ecosystem C allocation, mineral weathering and soil development on timescales relevant to human lifetimes.

Methods

Site and soil sampling description

We collected soil samples from the Calhoun Critical Zone Observatory, SC, USA, which consists of several research sites in the area of 34°36’25” N, 81°43’13”W. The region has a mean annual precipitation of 1250 mm yr^{-1} and mean average temperature of 16°C (NADP et al. 2017). These sites comprise paired, spatially replicated reference hardwood and regenerating pine forest plots that represent distinct land use histories. Reference hardwood forests are comprised of trees that are approximately 130 to 140 y old, dominated by *Quercus alba*, *Q. rubra*, *Q. montana*, and multiple *Carya spp.* Estimates of aboveground biomass in these forests averaged 21.1± 6.7 kg m^{-2} in 2014 (W. Cook and D. Richter, unpublished data). To the best of our knowledge, the soils in these forests have never been plowed or otherwise subjected to agricultural practices (Brecheisen et al. 2019). We compare these relatively old hardwood stands with regenerating pine stands, growing on land that was cleared for agriculture and cultivated for up to 150 y until its abandonment and regrowth both by natural reestablishment and by planting mainly of *Pinus taeda* and *P. echinata* from the 1930s through 1950s. The trees in these forests are approximately 60 to 80 y old, according to historical records of site abandonment. Estimates of aboveground biomass in these pine-dominated forests are statistically similar to those of the reference hardwood forests, averaging 14.6 ± 2.24 kg m^{-2} in 2014 (W. Cook and D. Richter, unpublished data), and modeling analyses indicate that biomass accumulation in regenerating forests has declined in recent decades (Mobley et al. 2015).

On May 1, 2018, we hand-augered five sampling cores in each of the two forest types at six fixed intervals (0-15 cm, 15-35 cm, 35-60 cm, 60-100 cm, 100-150 cm, and 150-200 cm) using a 10 cm diameter bucket auger. We homogenized soil collected in each of these depth intervals and collected a representative subsample. We froze samples as soon as possible and shipped them to the University of Kansas where they were maintained at -20°C except when briefly thawed for chemical analyses. These subsamples were then subjected to analyses for rooting system C exudates and soil P forms, detailed below.

Fine root abundances

To estimate differences across forest types in rooting depth distributions most relevant for nutrient acquisition, we quantified fine root abundances to two meters in soil profiles using the method described in Billings et al. (2018). For this analysis, we relied on soil data collected during the BigDig 2016 sampling campaign at the Calhoun, timed late in the growing season when root biomass was maximized. During the campaign, we dug ten >2 m deep soil pits and captured high-resolution photos along the length of each pit face that had been cleaned with a knife to expose natural planes of weakness. Six of these pits (i.e. three regenerating forests, three reference hardwood forests) are located in the same forest sites as hand-augered locations from May 2018. We then overlaid a 1x1 cm grid on each soil profile image using ImageJ software. In each 1x1 cm grid cell, we noted the presence or absence of roots, as well as its classification as fine or coarse, generating a measure of rooting density (units of roots cm^{-2}). We classified fine roots as roots < 1 mm in diameter, consistent with the idea that designating multiple size classes of roots < 2 mm in diameter is important for understanding root system functioning, and in acknowledgement of the importance of very fine roots for nutrient uptake (Pregitzer et al. 2002; McCormack et al. 2015; McCormack and Iversen).

Soil P stocks

Organic-, mineral-bound, and total P

We parsed organic P (P_o) and mineral-bound P (P_i) using the first two extractions of the Hedley soil P sequential extraction protocol (Tiessen and Moir 1993), which extracts nearly all P_o present in these soils (Richter et al. 2006), permitting us to divide total P estimates into an estimate of P_o vs. P_i in these soil samples. Briefly, we extracted 0.5 g of each soil sequentially with 30 mL 0.5 M $NaHCO_3$ followed by 30 mL 0.1 M NaOH. Each solution was shaken at 120 rpm for ~20 hr, centrifuged for 10 min at 3400 rpm, and filtered through a 0.45 µm polyvinylidene fluoride syringe filter prior to P analyses. We measured the P_i in these extracts according to the malachite green colorimetric method (D'Angelo et al. 2001) on a Bio Tek SynergyHT plate reader (VT, USA). We sent the remaining solution to Kansas State University to quantify the total P contained in these extracts via inductively coupled plasma – optical emission spectroscopy (ICP-OES, Varian 720-ES, Palo Alto, USA). The difference between values provided by the Kansas State testing lab and P_i in each extract provides an estimate of P_o in each solution, with the sum of P_o from NaOH and $NaHCO_3$ extracts approximating the total P_o in a given soil sample.

We also dried and pulverized five to ten gram soil subsamples for total soil P. We passed these subsamples through a 2 mm soil sieve and sent them to the Kansas State University Soil Testing Lab for total P analysis via salicylic-sulfuric acid digestion and ICP-OES analysis. The difference between previously summed P_o estimates and total P measured on dried, salicylic-sulfuric digested soils provides an estimate of overall P_i and P_o in each sample.

Bio-extractable P

To investigate the potential of rooting system exudates to generate bioavailable P from organic- vs. mineral-bound P sources, we extracted soils with acid phosphatase and oxalic acid solutions (DeLuca et al. 2015). We chose concentrations of each exudate well in excess of that found in a natural soil system based on trial extractions demonstrating greater P release with increasing extraction solution concentration. As such, P concentrations observed from these exudate-based extracts represent the maximum potential nutrient benefit to a plant with unlimited C allocation toward the given exudate.

For acid phosphatase (APase), we used purified APase (P1146, Sigma-Aldrich, USA) mixed with deionized water to make a solution with an enzyme activity approximately 1000 times that previously measured in Calhoun soils (Billings et al. 2018). We mixed this solution with soil samples in a 5:1 solution:soil ratio, agitated the soil solution for 30 minutes, centrifuged the solutions and filtered the resulting supernatant with 45 µm syringe filters. We then analyzed extracted liquid for orthophosphate P (PO_4^{-3}-P) on the microplate reader using the malachite green colorimetric method (D'Angelo et al. 2001).

To quantify oxalic acid extractable P, we made a 10 mM solution of powdered oxalate (129601000, Acros Organics, USA) and deionized water. We mixed the solution with each soil sample in a 5:1 solution:soil ratio and agitated samples for 30 minutes. After shaking, we centrifuged the samples and filtered the extracts with 0.45 µm syringe filters. Extracts were analyzed for PO_4^{-3}-P as above.

Evidence of rooting system exudates

As a means of estimating rooting system C investment in mineral vs. organic nutrient acquisition strategies, we analyzed rooting system exudate activities and concentrations in sampled soils. First, we examined extracellular APase activity—a representation of C investment in organic P acquisition—via protocols detailed in previous publications (Deforest 2009; Lehmeier et al. 2013; Min et al. 2014). We performed this analysis within two months of freezing soils given previously observed propensity for extracellular enzyme activity to decay in old samples (DeForest 2009). Briefly, we subsampled 1 g of each soil sample and mixed it with a sodium acetate buffer solution of pH ~5.5 for 30 seconds using a hand-held blender. We quickly transferred the solutions to black 96 well plates, to which we added either a fluorescently tagged phosphatase substrate (methylumbelliferyl phosphate, M8883, Sigma-Aldrich, USA), the fluorescent methylumbelliferyl label itself (MUB, M7008, Sigma-Aldrich, USA), or pH adjusted buffer to provide appropriate controls. The substrate, when cleaved by its relevant enzyme, releases a fluorescent signal that can be quantified as a metric of potential enzymatic activity. After plating, we incubated the plates for ~18 hr before analyzing the fluorescence at excitation wavelength 360, emission wavelength 460 on the plate reader (Lehmeier et al. 2013). All activities are reported in nmol g^{-soil} hr^{-1}.

We further transformed these potential activities into estimates of soil APase C concentrations to assess rooting system C investment in organic P acquisition. We divided the APase activity quantified in environmental samples (nmol g^{-soil} hr^{-1}) by the P we extracted from the same soils using a known amount of APase C (nmol g^{-1} hr^{-1} gC^{-1}), as described in bioextractable P forms above. This calculation reveals the maximum potential C in APase enzymes in these soils, given the greatest P benefit that these soils can contribute with no limitation of enzyme C addition. We further normalized these APase C concentration estimates by unit root abundance and assessed how these estimates varied with the fraction of total soil P present as organic P.

To assess rooting systems' propensity to use oxalic acid as a nutrient acquisition strategy, we extracted each soil sample by shaking soils with water (5:1 ratio) for 30 minutes. We centrifuged and filtered extracts with 0.45 µm syringe filters and froze them for subsequent analyses. We analyzed organic acid concentrations in bulk soils using ion chromatography. We conducted the analysis using an Ion Chromatography System (ICS) 2000 (Dionex,USA) fitted with Dionex IonPac AS11-HC 4 mm analytical and guard columns, following a modified version of the organic acid analytical protocol outlined by Thermo Fisher (2012). Briefly, we ran samples on a KOH eluent gradient of 1 mM, 15 mM, 30 mM, and 60 mM KOH, increasing concentrations at 15, 25, and 35 minutes respectively. Sample chromatogram peak areas were then analyzed using Chromeleon 6.8 Software build 2212. To determine retention time of oxalate anions, we compared chromatogram peaks produced by deionized water both with and without the addition of 5 µM oxalic acid. After we discerned the retention time, we produced analytical standards containing 0, 0.05, 0.1, 0.5, 1, and 5 µm oxalic acid using the Organic Acid Kit from Sigma Aldrich (47264) to determine oxalic acid concentrations in unknowns. We normalized oxalic acid C concentrations by rooting abundance metrics as for APase, and assessed how these normalized values vary with the inorganic fraction of total soil P.

Statistical Analyses

To assess the relative importance of forest history, soil depth and their interaction on root distributions, enzyme activities, organic acid concentrations, and all P fractions, we used linear mixed effects models. Two data sets, inorganic and organic soil P concentrations, required natural log transformation prior to linear mixed effect analyses to comply with model assumptions. For all response variables, we generated four statistical models: one with forest type as a fixed effect (1); one with depth as a fixed effect (2); one with both variables included as independent fixed effects but without their interaction (3), and one including all effects and their interaction (4) (Winter 2013). Models including depth as an effect were structured to account for the lack of independence across depths. In all models, we included sampling site as a random effect. We then ran ANOVAs between models 3 and 4 to test for a significant interaction. We interpreted a significant interaction between forest type and depth as a difference between forest types in the depth-associated pattern of the response variable; this approach embraces the lack of independence across depths and does not require post-hoc tests of the effect of forest type in each soil depth interval. When the interaction effect of forest type and depth on the response variable was insignificant, we tested models 1 and 2 against model 3 to assess the significance of forest type and depth individually as influences on the response variables.

As a means of assessing mechanisms of rooting system nutrient acquisition, we tested correlations between exudate C (i.e. APase and oxalic acid) per unit root and potential soil organic and inorganic P stocks both as concentrations and as proportion of total soil P, across all soil depths assayed in each of the ten plots. We log transformed metrics of exudate C concentrations per unit root to meet model assumptions. We used Cook's distance to check for outliers and influential points, and ran all analyses both with and without extreme points to examine their effects on tested relationships. We then tested for differences between the slopes of lines fitted to the five reference forest plots vs. the five regenerating forest plots via a paired t-test. The positive or negative nature of correlations between root exudate C metrics and potentially bioavailable soil P forms serves three roles: an index of rooting system C investment toward mineral vs. organic P acquisition; the extent to which those rooting system mechanisms differ with land use history; and the robustness of those observations across sampling space given the presence or absence of influential points.

Because fine root data exist at a much finer depth resolution (i.e. every 1 cm layer across 2 m) than the P fraction and exudate datasets (six fixed depth points), we used linear interpolation to generate values of P fractions. We considered this approach to be reasonable given monotonic trends with depth in P data. In one instance interpolations generated distinct clusters of points where the examined soil profile characteristic varied greatly between sites within a given forest type. We considered these interpolated profiles as replicates and the calculated variability as part of a standard error. We then performed correlation analyses between root density and measured P fractions across soil depths and forest types as a means of inferring root abilities to acquire P from the measured P pools. Where correlations exhibited non-linear relationships, we fitted logarithmic, power law, and exponential equations to the data and selected the model with the lowest AIC value as the best representation of the relationship. In those cases, *P*-values representing significance reflect rejection of the null hypothesis that there is no relationship between X and Y variables.

Errors presented are one standard error of the mean. We report all P values suggesting meaningful trends in the data (Amrhein et al. 2019). All analyses were run using R via R Studio, Version 1.0.153 (RStudio Team, 2017) with packages lme4 Version 1.1-21 and ggplot2 Version 3.1.1 (Bates et al., 2015; Wickham, 2016).

Results

Fine Roots

Forest type and depth both exhibited a significant influence on fine root abundances in these forests. Reference hardwood forests had higher fine root densities across soil profiles than regenerating forests (*P*=0.047, Fig.1). The fraction of fine roots in each 1 cm soil depth interval declined exponentially with increasing soil depth in both forest types (*P*=0.0001). Reference hardwood fine root abundances declined from 49% to 4% and regenerating pine root abundances declined from 43% to 1.5% in the top 1 cm to 200 cm, respectively. The legacy of land use history in metrics of fine root density is particularly evident one meter deep in the soil profile, a point below which we observed far smaller abundances of fine roots in both forest types but consistently greater abundance of fine roots in reference forests, approximately double that in regenerating forest profiles.

Soil Phosphorus Pools

There was a significant interaction between forest type and depth driving inorganic P concentration patterns in Calhoun soils (*P*=0.03, Fig 2a). In reference forests, inorganic P showed little variation in concentration across depths and sites (Fig. 2a), with an average of 82 mg kg^{-1} across soil samples. In regenerating forest soils, we encountered more inorganic P on average (100 mg kg^{-1}) than in reference forests, but there were less consistent inorganic P concentration patterns when compared with reference forest P depth distributions. Each regenerating site has a distinct land use history, likely producing high variability in P concentrations across regenerating forest sites. The large standard errors (Fig. 2a & b) and range of inorganic P values from 1.33 mg kg^{-1} to 496 mg kg^{-1} across all regenerating forest soil samples highlight this subsurface variability in P metrics. Organic P concentration declined significantly with depth in both forest types (*P*= 0.0003 Fig. 2b), having high concentrations in the soil surface declining to near-zero values in deep soils.

In general, inorganic P comprised an increasingly larger proportion of the total P with soil depth (*P*=1.17e-06, Fig. 2c), and made up a larger fraction of the total P than organic P in all except the shallowest soil horizons across forest sites (Fig. 2c & d). Correspondingly, organic P comprised an increasingly smaller proportion of total P with increased soil depth (P = 1.17e-06, Fig. 2d) in both forest types.

Potential linkages between P stocks and rooting system activities

Root distribution and bioavailable P

Below 100 cm in Calhoun regenerating forests, fine roots occupied at most 1.5% of each 1 cm soil depth interval. Although few in number, fine roots in reference forests were nearly double that of regenerating forests, hinting at potential disparities in trees' resource-acquiring activities in these deep soils. Indeed, within the depth interval just above this depth (60 to 100 cm), we observed differences in the relationships between fine root abundances and potentially bioavailable P stocks. Across forests, this depth interval represented part of the clay-rich Bt horizon although was nearer the C horizon in regenerating forests than reference forests (W. Cook and D. Richter, unpublished data). Thus, this interval in both forests is a potential zone of material accumulation, but regenerating fine roots may not have to extend as deeply before reaching less mineralogically-altered material. In these forests, oxalate-extractable P, a metric of potential bioavailable mineral-bound P, declined where roots were more abundant (Fig. 3a, P = 0.0002). Acid phosphatase-extractable P, a measure of potentially bioavailable organically-bound P, also exhibited a negative trend with fine root abundance (Fig. 3b, P = 0.0002).

In this same depth interval in reference forests, where fine roots were relatively more abundant, oxalate-extractable P was present only in soils where we observed few to no roots (Fig. 3a, P=0.001). Unlike regenerating forest soils, reference forest fine roots displayed a positive relationship with APase-extractable P (Fig. 3b, P< 0.0001) suggesting that organically-bound P remained relatively abundant in the presence of roots in these same soils.

Potential rooting system exudate production

In both forest types, oxalic acid-C concentrations per unit root abundance tended to be greater where inorganic, mineral-bound P comprised a greater proportion of the total soil P content (*P*=0.058 and 0.07 for reference and regenerating forests respectively, Fig. 4a & c). In contrast, estimates of phosphatase-C per unit root abundance were greater where organic P comprised a relatively small proportion of soil P stocks, in both forest types ($P = 6.8e^{-8}$; P =0.0003, Fig. 4b & d). In both cases, we observed no significant difference in the slope of the lines representing the exudate vs. soil P relationship in regenerating forests when compared to reference forests, although the absolute concentration of both exudates produced per root was consistently greater in regenerating forests compared to reference forests. In both exudate datasets, we encountered up to three outliers as defined by Cook's distance test for influential points. The APase dataset contained one outlier in reference forests and three in regenerating forests. For oxalic acid, we observed two outliers in each forest type. These points generally represented soil samples in which APase or oxalic acid concentrations were below detection limits and thus generated an unrealistic fit to otherwise linear, log-transformed data. We tested the relationship between X and Y with and without these influential points; in most cases, these analyses revealed significant relationships between root responses and P forms when outliers were omitted, but not when outliers were included. One dataset, oxalic acid per unit root vs. proportion inorganic P (Fig. 4c) did not exhibit a change in significance regardless of outlier inclusion. We report results both with and without outliers in Fig. 4.

Discussion

This study illuminates persistent anthropogenic legacies deep within Earth's CZ even after six to eight decades of forest regeneration on post-agricultural land. These legacies are manifest most obviously as truncated fine root depth distributions (Fig.1) and altered depth distributions of P nutrient stocks (Fig. 2). These two attributes appear to drive subtle shifts in forest functioning that nevertheless represent persistent legacies of anthropogenic land use that influence these forests' C economies. Because fine roots are plants' primary methods of acquiring resources (Jackson et al. 1997; Liu et al. 2015; Kong et al. 2016), any redistribution of fine roots resulting from anthropogenic disturbances both represents and necessitates shifts in forest mechanisms of obtaining nutrients. Land use history may further promote changes in forest nutrient acquisition strategies by altering the nutrient stocks those fine roots can encounter as trees reestablish. Here, we provide one, place-based example that supports our hypothesis that changes in rooting system development and nutrient stocks incurred as a result of historic land use alter the depths at which, and mechanisms with which, forest ecosystems acquire nutrition. We propose that shallower rooting system depths in regenerating forests compared to older, reference forests may promote distinct C for P tradeoffs reflective of divergent nutrient economies (Fig. 5).

Carbon investments: Rooting systems display depth-dependent C allocation strategies to acquire nutrition

Consistent with our first hypothesis, rooting system C distribution in these forests' soils reflects time since disturbance and the nutrient forms present in the rooting zone. Foremost, more rooting system C is present in the form of fine roots more deeply in the reference forest soil profiles than in the regenerating forests (Fig. 1). This phenomenon likely results from several factors. First, regenerating rooting systems have had only ~80 y to establish compared to well over 100 y in reference forests and may not have had the time needed to develop such extensive networks (Knops and Bradley 2009; Yuan & Chen, 2012; Pierret 2016). In addition, succession-associated tree species differences—early successional pines in regenerating forests and late successional oaks in reference forests–typically exhibit different rooting structures (Shukla & Ramakrishnan 1984; Finér et al. 1997; Lambers et al. 2008), a feature that emphasizes another way in which time since disturbance can contribute to belowground root abundances and functioning. Though regenerating rooting systems could eventually extend to comparable depths as reference forests, there may be limited incentive for roots in regenerating forests to grow into deeper soils because surface soils accumulate organic matter in the reestablishing O horizon in the early years of forest regrowth (Mobley et al. 2013) that re-growing roots can access as a source of relatively abundant nutrients (Fig. 2. Crews et al. 1995; Cross and Schlesinger 1995; Vitousek et al. 1997). This may be particularly relevant for species such as the CCZO's loblolly pines, which invest significant C stores in ectomycorrhizal fungi that produce exudates—including phosphatase—at relatively high rates (Lambers et al. 2008; Yin et al. 2014). Though potentially expensive, a shallow C allocation strategy for nutrient acquisition might be resource-effective for these early successional, shade intolerant pines that can invest C in nutrient acquisition, but have not yet allocated C to deep root construction (Phillips et al. 2013).

Rooting systems in these forests also provide clues of depth-dependent C allocation strategies other than fine root distributions: their rooting system exudation patterns appear to reflect organic vs. mineral nutrient stocks in the rooting zone. Across soil depth, rooting systems appear to allocate more C per unit root to oxalic acid generation where inorganic P stocks comprised a greater proportion of total soil P, regardless of forest type (Fig. 4a & c). Numerous authors demonstrate that oxalic acid liberates P from mineral-bound forms while requiring relatively little plant C to produce (Aoki et al. 2012; Keiluweit et al. 2015; Lugli et al. 2019).

Thus, our results suggest that roots may leverage relatively low C-cost nutrient stocks when possible to optimize nutrient returns on their resource expenditures.

In contrast, APase concentrations per root were high only when relatively little organically bound P was present in the rooting zone, regardless of forest type (Fig. 4b & d). Numerous studies have observed rooting system phosphatase production in soils primarily where P is relatively scarce (Bunemann et al. 2011), which may be a function of the high C-cost of enzyme exudates and mycorrhizal partners, both of which can mobilize organic nutrients. Andrino et al. (2019), for example, demonstrate that rooting systems without mycorrhizae are less able to acquire organically-bound P as well as inorganically-bound P. However, they demonstrate that mycorrhizae, which enable plant access to organic P stocks they might not otherwise obtain, confer a smaller net P benefit for plants' C investments than inorganic P (Andrino et al. 2019). Thus, C allocated to organic nutrient acquisition likely is beneficial in soil conditions where organic nutrient stocks are the prevailing nutrient source, as in shallow horizons or highly chemically denuded soils. Our study suggests that rooting systems appear to invest in these C-expensive mechanisms only when necessary, such that nutritional benefits should exceed high C costs.

Because depth distributions of SOC influence forest soils' capacity to retain C over decades to centuries (Rumpel and Kogel-Knaber 2011), distinctions among forests' strategies of belowground C allocation for nutrient acquisition may contribute to longer-term belowground C budgets. Roots themselves represent one of the largest contributors to soil C stocks, with increasing relevance to soil C accumulation in deeper soils (Rasse et al. 2005; Crow et al. 2009). Further, their exudates are implicated in soil C retention (Rasse et al. 2005; Keiluweit et al. 2015). Indeed, some studies hint that soil retention of C-rich exudates may exceed C lost as CO_2

due to mineralization of SOC induced by those exudates (Qiao et al. 2013). Exudate fate depends on its identity, however. Exo-enzymes, for example, likely enhance CO_2 release due to their specific role as catalysts of organic matter decay and thus as provisioners of microbially available nutrient and C resources (Schlesinger 1984; Scheckenberger et al 2008). Organic acids, on the other hand, have a high propensity for adsorption onto Fe- and Al-minerals (Rasse et al 2005; Ganor et al. 2009) and the ensuing ligand exchange process can result in soil retention of this exudate while simultaneously displacing nutrient-containing ions into soil solution (Ganor et al. 2009; Keiluweit et al. 2015; Hasegawa et al. 2015). Rooting systems leveraging this exchange for nutrients may mineralize relatively less C to CO_2 than an exo-enzyme induced transaction, given that nutrients are made available via C-binding ligand exchange and not by microbial activities that incur a respiratory cost (Schlesinger 1984; Rasse 2005). Indeed, Richter et al. (1999) observed net C loss in regenerating forest soils during 40 y of rooting system development. We cannot know the degree to which these phenomena drive SOC depth distributions in these forests, but it seems reasonable to infer that distinct patterns of vegetation C exchange for different P forms may influence SOC generation and retention.

Depth-dependent P acquisition strategies reveal persistent legacies of land use

These data are also consistent with our second hypothesis. Potential rooting system P acquisition from mineral vs. organic stocks appears to vary with both depth and forest type because historic agricultural practices have redistributed plants' primary mechanism to obtain nutrients – fine root proliferation – relative to distributions of nutrient forms (Figs. 2, 3). Fine root proliferation in these regenerating soils is approximately half that of reference forests below ~100 cm (Fig 1). Just above this depth (60-100 cm), reference and regenerating rooting systems appear to employ different nutritional strategies, exhibiting apparent uptake of the distinct P forms present in the rooting zone of each forest type. In reference forests, roots between 60 and 100 cm appear more successful at acquiring inorganic P compared to organic P stocks (Fig. 3). Roots in regenerating forests in this same depth interval, though relatively few, appear to deplete both organic and inorganic forms (Fig. 3).

This distinction in P resource use between forest types likely reflects the interaction of altered fine root distributions and distinct abundances of P stocks in the rooting zone of each forest type. Reference forests display relatively less variable inorganic P concentrations across soil depths compared to regenerating forests (Fig. 2a). This implies that, in these older forests, investment in low C-cost oxalic acid would be a reliable mechanism for rooting system P acquisition (Fig. 3a), given that these mechanisms promote P release from mineral-bound P forms (Fig. 5. Crews et al. 1995; Keiluweit et al. 2015; Hasegawa et al. 2015; Ellsworth et al. 2017). Like reference forests, regenerating forest roots encounter both organic and inorganic P, but these stocks are more variable across depths and sites (Fig. 2). Reduced predictability of soil P stocks might explain the appearance of an "omnivorous" or opportunistic nutrient strategy in regenerating forests, reflecting the benefits of synergistic nutrient acquisition strategies where P stocks are variable (Fig. 5. Marschner and Rengel 2007; Darch et al. 2016; Brantley 2017; Lugli et al. 2019). Given that these regenerating forests appear thus far to have constructed fewer fine roots between 1 and 2 m than in the reference forests, they may have more C resources to spend on C-costly extracellular enzymes than reference forest trees, either directly or through mycorrhizal fungi, promoting shallow soil P for C exchanges in place of deep mineral-P uptake.

Conclusions

Our results suggest that rooting systems develop depth-dependent C distribution patterns in response to organic vs. mineral bound nutrient stocks, perhaps optimizing the nutrients plants obtain for the C resources they invest. Further, the relative availability of organic vs. mineral nutrient stocks reflects historic land use, prompting anthropogenic legacies to produce distinct ecosystem nutritional economies. These distinct economies have implications for soil C fate. Deep rooting systems in relatively old forests represent nutritional economies that can sequester root C and root exudate C meters deep into Earth's surface. Forests regenerating post-disturbance, in contrast, exhibit nutritional strategies focused on relatively surficial horizons' nutrient stores for decades post-disturbance. In these forests, the relative dearth of deep roots and the C-rich, exo-enzyme dominated mechanisms of liberating surficial, organically-bound nutrient stores emphasize surface-focused strategies for nutrient acquisition. The demonstration of the multiple ways in which reference forests contain and possibly retain C more deeply in the subsurface compared to regenerating forests adds support to propositions that older forests may continue to serve as a net C sink well after aboveground biomass appears to reach steady-state (Magnani et al. 2007; Baldocchi 2008; Luyssaert et al. 2008). Similarities in regenerating and reference forests' aboveground biomass in this study suggest that aboveground growth can mask lags in rooting system development in regenerating forests that can inhibit their capacity to establish the C sink and SOC retention mechanisms present in older forests. Distinctions across rooting system economies likely have meaningful implications for soil development via both mineral weathering and C cycling dynamics on time scales relevant to human lifetimes, representing subtle but consequential signals of the Anthropocene.

Figures

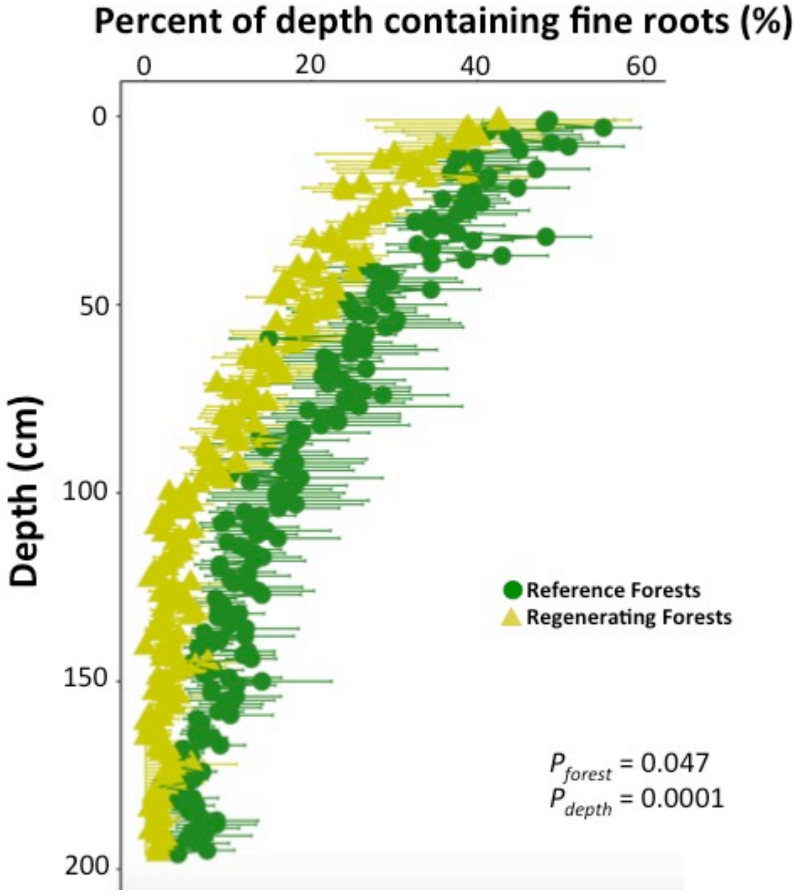

Figure 1. Fraction of each 1-cm depth interval containing fine roots (< 1 mm diameter) to 200 cm depth in reference and regenerating forest profiles at the Calhoun CZO, SC, USA. Each data point represents the average of five sites. Error bars represent one standard error of the mean. See Statistical Analyses in Methods section for explanation of the model used to generate *P*-values.

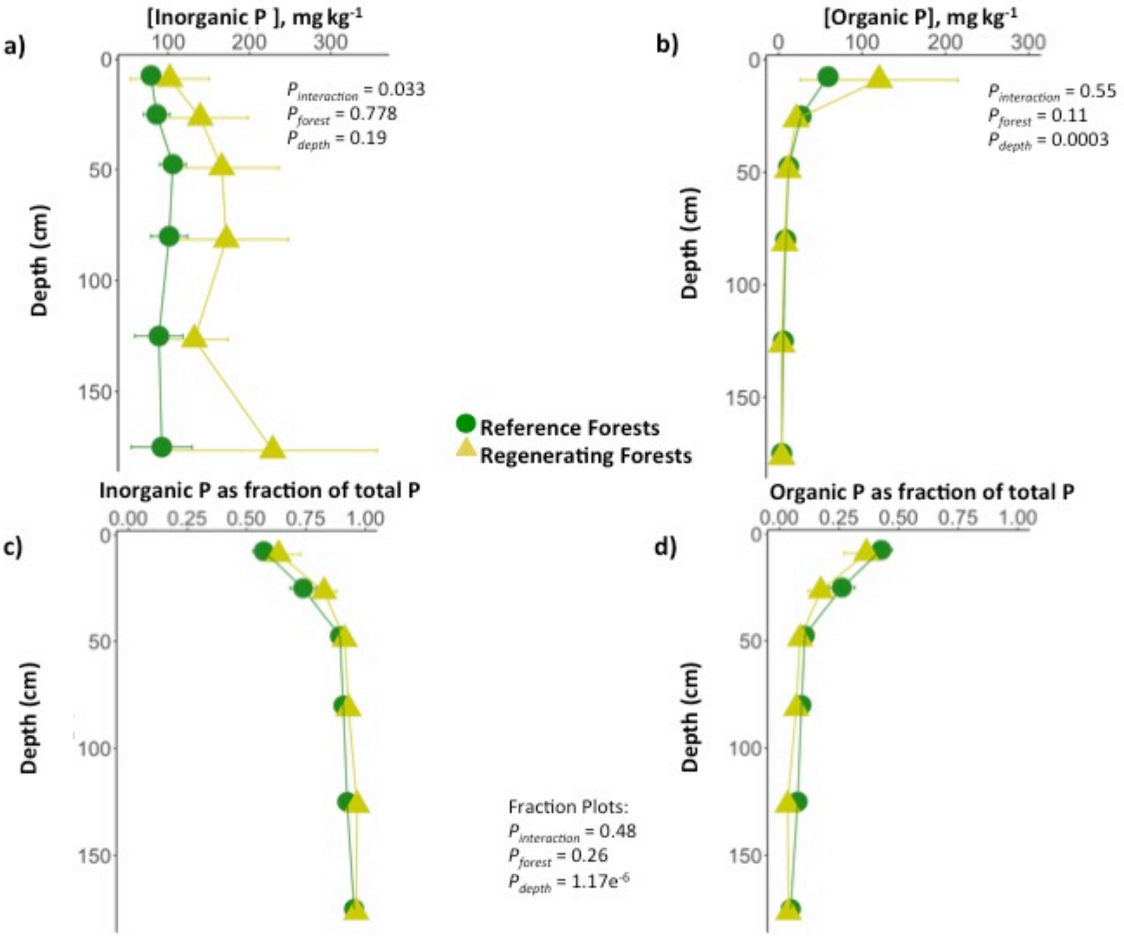

Figure 2. Soil P stocks to 200 cm in regenerating and older reference forest soils at the Calhoun CZO, SC. Each data point represents the mean of five sites. Error bars represent one standard error of the mean. Pools include a) mineral-bound NaOH- and $NaHCO_3$-extractable Hedley P fractions; b) organic P stocks estimated via the difference between salicylic acid-digested total P and inorganic P in (a). We also examined proportion of total P comprised of c) inorganic P and d) organic P across depths and sites. Note that statistical results for (c) and (d) are identical given that organic P pools are derived using inorganic P pool sizes, and that soil depths for regenerating forests have been offset by 1.5 cm for error bar visualization. See Statistical Analyses in Methods section for explanation of the model used to generate *P*-values.

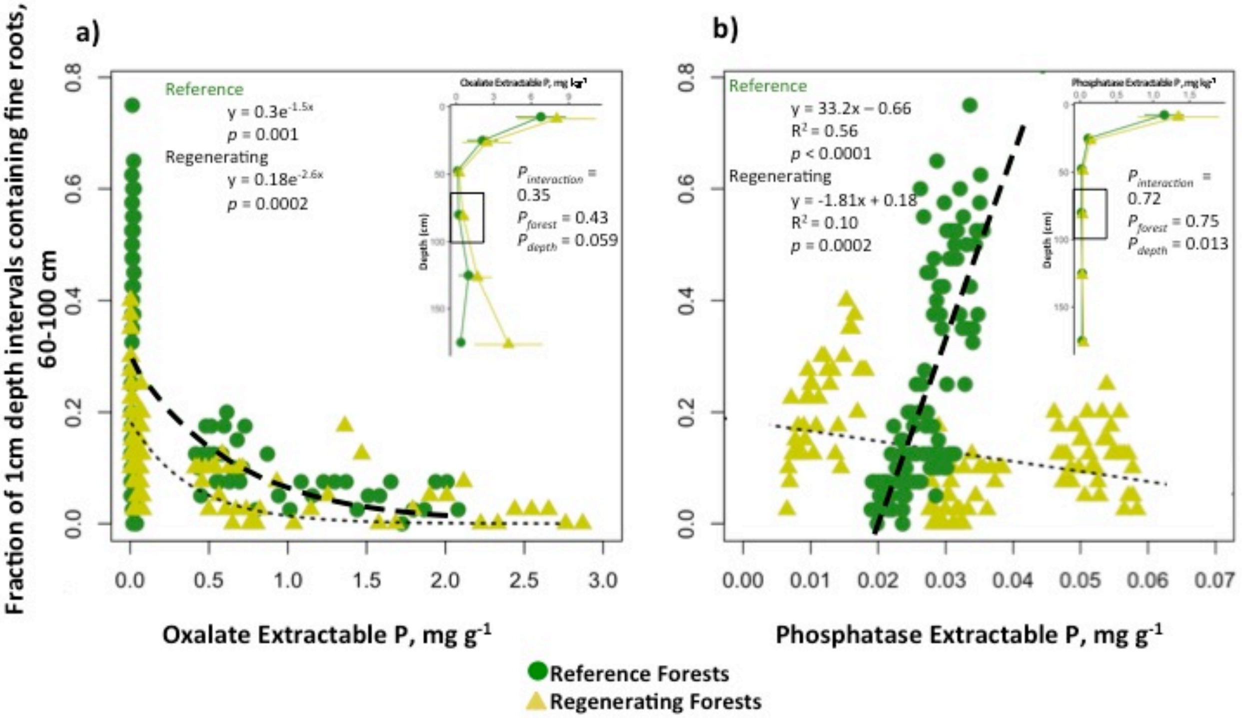

Figure 3. Fraction of fine roots in 1-cm depth intervals vs. soil P extracted with synthetic root exudate solutions comprised of a) 10 mM oxalic acid and b) sufficient APase to generate an activity of 100 nmol g^{-1} hr^{-1} in samples collected from 60-100 cm deep soils in CCZO forests. Inset plots show P concentrations obtained from a) oxalate and b) phosphatase extraction across all soil depths in these forests, with black boxes denoting the sampling region represented by interpolated data points in main plots. Data clusters are an artifact of interpolation. For further explanation, see Methods

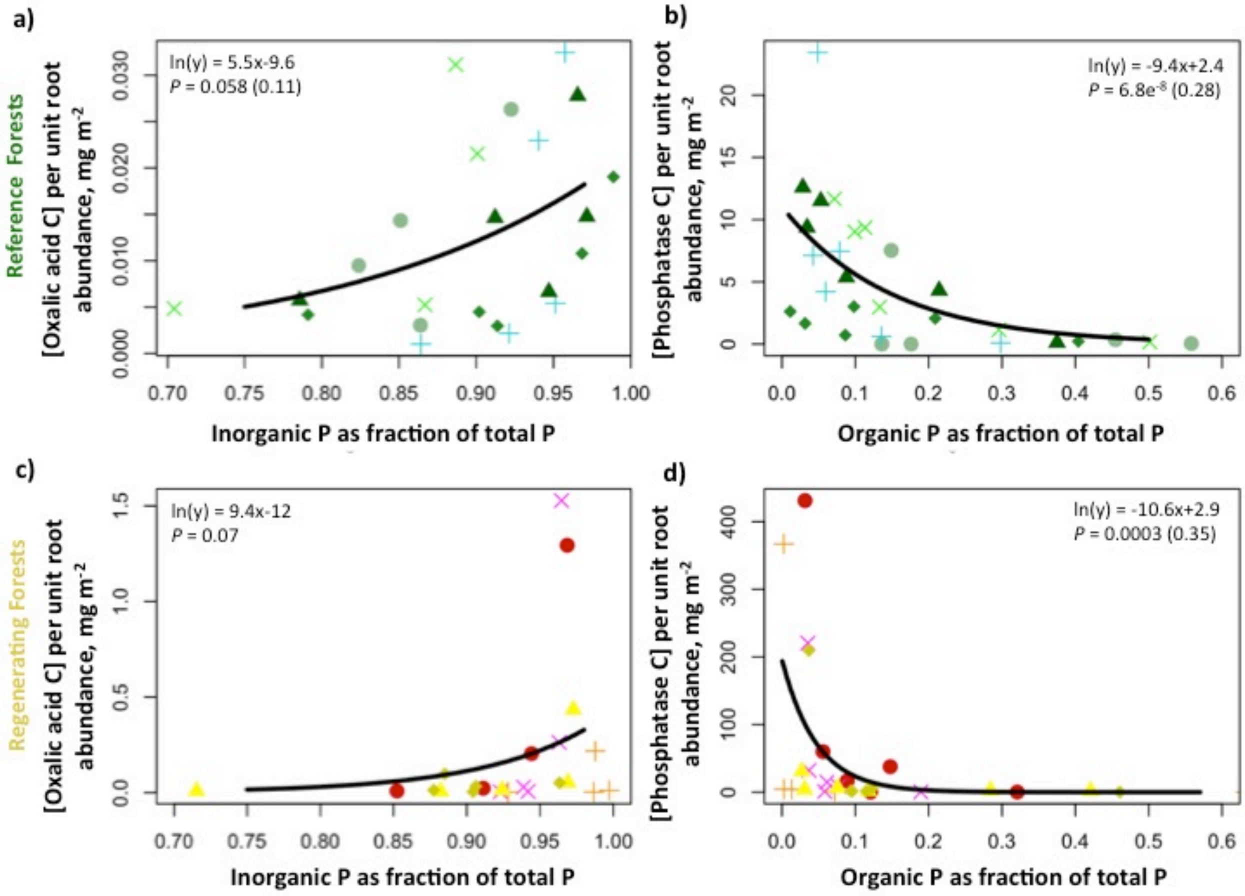

Figure 4. Rooting system exudate concentrations as they relate to inorganic and organic P proportions of total P across all depths in CCZO forest soils. In reference forests, a) oxalic acid per unit root abundance in each depth increment vs. inorganic proportion of soil P, and b) acid phosphatase per unit root abundance in the soil depth increment vs. organic proportion of soil P. c) and d) represent analogous data sets for regenerating forests, respectively. All plots depict untransformed data with outliers removed. Symbols of the same shape represent paired reference and regenerating sites. Black lines depict the average fitted trend across all data points. P values are reported on log-transformed data both with three outliers (values in parentheses) and without outliers, where outlier inclusion produced statistical differences. Reference and regenerating forests displayed no significant differences in the slope of fitted curves for the same relationship (i.e. a vs. c, or b vs. d), but note greater absolute exudate production in regenerating forests highlighted by y-axis scales, which are different across all panels to highlight trends.

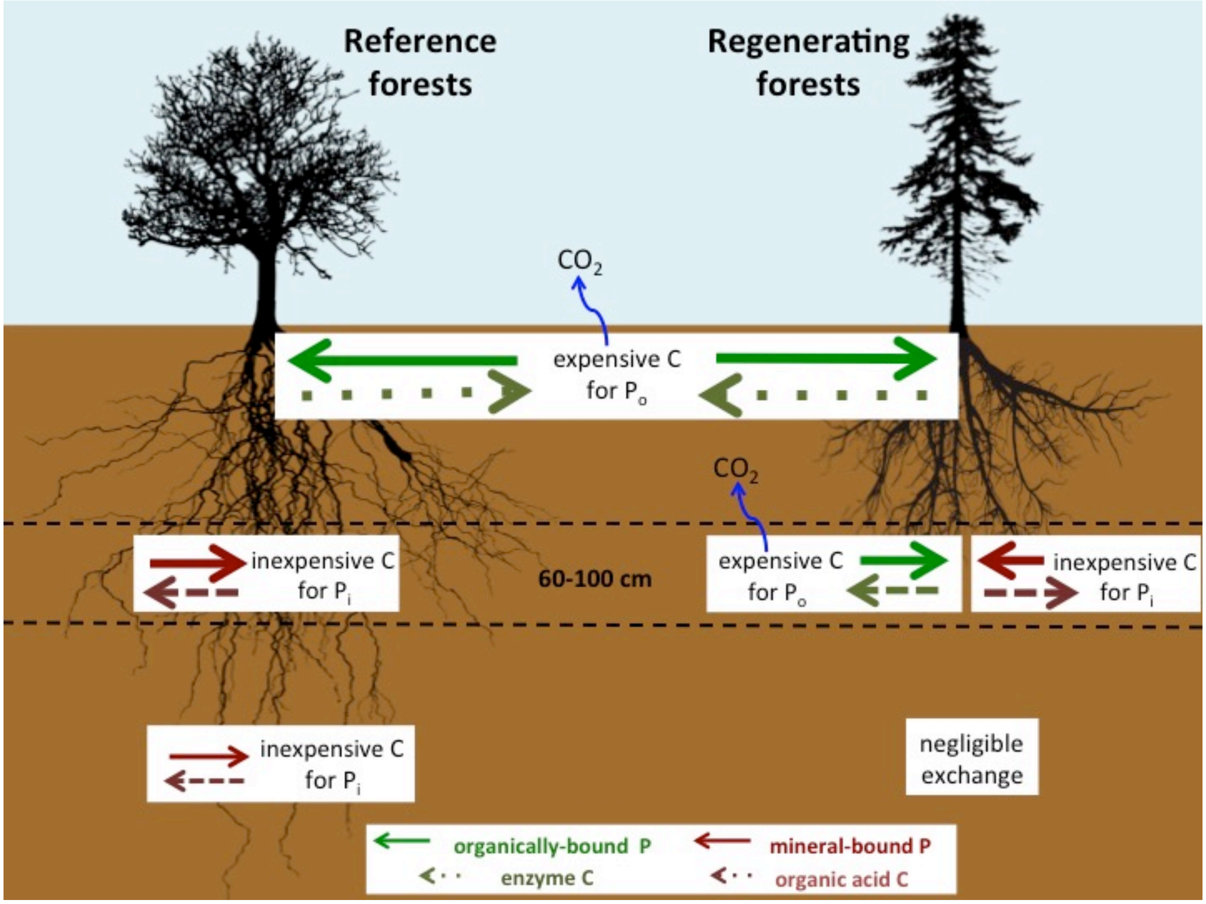

Figure 5. Conceptual representation of rooting system nutrient acquisition economies in regenerating forests compared to their older, reference forest counterparts. We depict three distinct belowground C for P exchange regions: a shallow exchange in both forest types governed by C-rich enzyme exudate-driven organic matter decomposition; a region from 60 to 100 cm where rooting systems in regenerating forests diverge from reference stands, accessing both mineral- and organic matter-bound P; and a deep exchange region governed by low-C organic acid-induced mineral-bound P release, occurring only in older reference forests. Arrow length and thickness represents the degree to which each exchange plays a role in a rooting system's nutrient economy. The CO_2 feature associated with enzyme C exchange for P_o refers to microbially-mediated CO_2 losses typically linked to organic matter decay, a phenomenon that mineral P release does not necessitate

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Chapter 4: Adaptable economies: Plants maximize C exchange for distinct P forms across soil depth

Abstract

Plants invest C belowground in part to capture nutrients that vary in form and abundance over time. Plant nutrient acquisition strategies can redistribute soil nutrients via nutrient uplift, making them important mechanisms underlying the development of Earth's critical zone. Developing a predictive understanding of these C-for-nutrient exchanges is critical to projecting future soil organic C stocks and vegetation's nutrient sources with environmental change. Nutrient forms tend to follow predictable trajectories across space and time. One of the leading concepts describing these patterns was proposed by Walker and Syers (1976) for P, which transitions from dominantly mineral-bound to dominantly organically-bound forms over millennia. The rooting distributions and C compounds required to liberate nutrients from organic materials is different from those most effective for releasing nutrients found in rocks and minerals, consistent with our previous pedon-scale observations of vegetation altering belowground C allocation as nutrient sources change. Here, we test the hypothesis that rooting systems exhibit plasticity in C resource allocation that results in P uptake under different nutrient distribution scenarios, and that plants' C-for-P exchange mechanisms are sufficiently plastic to reflect organic vs. mineral nutrient sources regardless of soil depth. We conducted a greenhouse experiment in which different combinations of organic- and mineral-bound P at varying depths were offered to vegetation. Over the course of the experiment, P accumulated in roots and shoots and declined in soil organic P pools, suggesting that organically-bound P served as the source of the most bioavailable P form in this system. Rooting systems allocated C to root proliferation and acid phosphatase production in soils containing organically-bound P, regardless of its depth

in the soil profile, demonstrating that plants are capable of altering their C allocation in ways that capitalize on this bioavailable P form regardless of where they encounter it in the profile. It is possible that free-living microbes may have produced the phosphatase activity we observed, but that activity may have resulted in neighboring roots exploiting microbially released P. These rooting system C-for-P exchanges generated evidence of nutrient uplift in soils where organic P was distributed deeply, the first direct observation of plants performing this nutrient redistribution activity. Our findings reveal ways plants may cope with anticipated shifts in rooting distributions and soil nutrient availability in the Anthropocene and demonstrate the mechanisms by which vegetation drives both the C and nutrient dynamics throughout Earth's critical zone.

Introduction

The heterogeneity of soil nutrients available to terrestrial vegetation prompts countless questions surrounding ecosystem nutrition and the sustainability of nutrient sources over time (Richter and Markewitz 2001; Hodge 2004; Porder and Ramachandran 2012; Hauser et al. *in review*). Such uncertainties are important to address because nutrient bioavailability influences key ecosystem functions such as plant growth. Perhaps less obvious, nutrient bioavailability may dictate patterns of soil carbon (C) distribution because plants allocate some of their C resources to root-driven processes that promote the release of nutrients bound in different forms (Lynch and Ho 2005; Lambers et al. 2008; Lugli et al. 2019; Hauser et al. 2020). Plant C allocated to these functions can be exuded directly from roots, or indirectly via mycorrhizae; either way, it represents plant C allocated to nutrient acquisition. Because mycorrhizal associations are critical to nutrient acquisition for most plants (Bolan 1991; Hodge 2004; Bunemann et al. 2011, Liu et al. 2015) here we consider roots and mycorrhizae to comprise plants' rooting systems (Doussan et al. 2003; Yan et al. 2006; Wang et al. 2009; Brearley 2011; Billings et al. 2018; Hauser et al. 2020), and thus consider the C and nutrient dynamics driven by those rooting systems as an integrated system. As such, illuminating vegetation strategies of nutrient acquisition that rely on rooting system proliferation and exudation of C-rich compounds (referred to here as rooting system exudates) can help us gain a physiological understanding of patterns of plant C allocation and ultimately a predictive understanding of ecosystem-scale C fate.

One likely driver of variability in rooting system C allocation is the changing relative abundance of mineral- vs. organically-bound nutrients among and throughout soil profiles. As soils develop over millennia, their mineral nutrient content tends to decline, while the presence of organically-bound nutrients tends to increase during decadal-scale ecosystem development on those same soils (Vitousek et al. 1997; Crews 1995; Hauser et al. *in review*). These same nutrient forms change across soil depth, with more organically-bound nutrients typically found in shallower soil horizons and more mineral-bound nutrients found in deeper soils where mineral surfaces tend to be less weathered (Jobbagy and Jackson 2001; Brantley et al. 2012). As nutrient forms change over time and space, the C expenditure required for rooting systems to access those nutrients also changes (Cross and Schlesinger 1995; Lugli et al 2019; Peixoto et al. 2020; Hauser et al. 2020), likely because distinct rooting system processes are associated with different C costs. In part because of the mismatch of timescales relevant for these mineral and organic nutrient cycling processes (Drever 1994; Pedersen and Bille-Hansen 1999; Kavvadias et al. 2001; Brantley 2008; Ferrier et al. 2010; Hauser, in review), understanding how these patterns of belowground C allocation drive ecosystem nutrient dynamics has proven difficult.

Many studies exploring these issues (*e.g.*, Crews 1995; Vitousek et al. 1997; Menge et al.

2012; Hauser et al., *in review*) are guided by a now-classic hypothesis about changing phosphorus (P) availability in ecosystems over time (Walker and Syers 1976). The focus on P stems from its low supply in many systems relative to its high demand, and its status as a nutrient derived solely from rock minerals until incorporation into organic matter (Bunemann and Condron 2007; Schlesinger and Bernhardt 2013). Plant promotion of nutrient bioavailability typically requires diversion of fixed plant C to a diversity of rooting system exudates (Bunemann et al. 2011; Fujita et al. 2017). Thus, it is logical that patterns of plant P uptake are linked to belowground C-allocation strategies as they vary across space and time. Indeed, observed patterns of root C, rooting system exudates, and distributions of P pools in a forested ecosystem point to such linkages and suggest that trees fine-tune rooting system C investments to those most appropriate for the nutrient resources surrounding them (Hauser et al. 2020). Specifically, plants appear to invest in low C-cost organic acid exudates where roots are deeply distributed and are more likely to encounter P-containing mineral surfaces, and C-rich exudates—like acid phosphatase that releases plant available phosphate from organic molecules (Bunemann and Condron 2007; Buneman et al. 2011)—where roots are shallowly and abundantly distributed in relatively shallow, organic-P rich soil horizons (Hauser et al. 2020). Additionally, plants have been observed allocating C to enhanced root growth in the presence of organic-matter-bound P, suggesting that root proliferation in organic P patches is beneficial for plant nutrition (Li et al. 2010). These findings are consistent with recently conceptualized plant nutrient economic frameworks that suggest that rooting systems develop sets of mechanisms targeting mineral-bound and organically-bound nutrient sources (Lambers et al. 2008; Phillips et al. 2013) and that these plant strategies may change with soil depth (Peixoto et al. 2019).

As a result of these distinct rooting system strategies targeting specific nutrient forms, plants influence metrics of soil development (Jobbagy and Jackson 2001; Rasse et al. 2005; Pierret et al. 2016; Austin et al. 2018; Cui et al. 2019; Koop et al. 2020), some of which reveal patterns consistent with nutrient uplift—the process by which plants' rooting systems tap into deeply distributed soil nutrients and recirculate them in shallower surface soils as plant materials senesce and decay (Jobbagy and Jackson 2001; Austin et al. 2018). Developing clarity in our observations of nutrient uplift is important for identifying how and where nutrient resources are distributed in soils and for making inferences about biotic influences on Earth's critical zone (CZ, Brantley et al. 2012; Dontsova et al. 2020). Nutrient uplift is typically inferred based on soil nutrient distributions that evolve over long timescales (i.e., millennia; Jobbagy and Jackson 2001; Brantley et al. 2012), but direct observations of plants performing this process have not been reported. As a consequence, conclusions about plant strategies for organically- vs. mineral-bound nutrient sources also tend to be inferred given the long timescales over which soil development metrics emerge (Drever 1994; Brantley 2008; Brantley et al. 2012), frequent focus on observations made at the ecosystem scale (i.e., Hauser et al. *in review*), and the relative rarity of ecosystem-relevant experimental manipulations.

A true test of plants' ability to alter their C allocation strategies to obtain organic- vs. mineral-bound nutrients, and thus perform nutrient uplift in the presence of deeply distributed bioavailable nutrients, would be to present rooting systems with inverted soil profiles. For example, planting seedlings in soils containing mineral-bound P layered over organically-bound P would require roots to allocate C to processes typical of shallow soil horizons in deep soils to obtain the same P benefits. However, studies that have explicitly quantified the degree to which the relative abundances of various rooting system C pools (*i.e.*, roots and exudates produced) reflect varied abundances of mineral vs. organic nutrient pools, or the degree to which plant C allocation strategies can change as the presence of these distinct nutrient forms changes across soil depth have not been conducted in controlled, replicated settings. As a result, the degree of plasticity in rooting system strategies for nutrient acquisition as mineral- vs. organically-bound resources vary remains unclear, and our understanding of the influence of any such plasticity on processes such as nutrient uplift (Jobbagy and Jackson 2001; Brantley et al. 2012; Austin et al. 2018) is hindered. This lack of understanding results in negative consequences for our ability to project vegetation functioning in the Anthropocene, a period likely to produce changes to plants' belowground C and nutrient tradeoffs due to changing root depths (Hauser et al. *in review*) and changing availability of nutrient forms due to fertilization and accelerated erosion (Amundson and Jenny 1991; Haff 2010; Yoo and others 2015).

To help fill this knowledge gap, we conducted a greenhouse experiment in which we grew seedlings in a medium containing either inorganic or organic P, or some depth-dependent combination of the two. We quantified variation in rooting system C investments — including root construction and exudate production — in response to the changing relative abundances of mineral vs. organic P pools across time and depth, and the degree to which plant P acquisition benefited from plant C investments. We hypothesized that if plant belowground C allocation strategies are sufficiently plastic to exhibit nutrient acquisition strategies specific to the availability of nutrient forms, rooting systems would demonstrate distinct P uptake and C allocation strategies congruent with relative abundances of organically- and mineral-bound P regardless of the depth at which the nutrient source is located in the soil profile (Fig. 1). For example, we would expect to observe a high degree of root proliferation and rooting system investment in extracellular phosphatase in organic matter-rich soil horizons regardless of the depth of those organically-bound resources. We also would expect signals of nutrient uplift

(Jobbagy and Jackson 2001; Brantley et al. 2012; Austin et al. 2018) to be expressed as enhanced abundances of P in shallowly distributed plant P pools and depleted abundances of P in deeper soils, regardless of its form, over time. Such a phenomenon would be consistent with shifting nutritional strategies as roots deepen and encounter different nutrient forms. Our hypotheses explicitly probe the observation that plants exert some degree of control over soil P acquisition (Bunemann et al. 2011; Hauser et al. 2020), either directly via root exudates or indirectly via mycorrhizal exudates, and thus influence the depth distribution of P forms. This work thus attempts, for the first time, to directly observe nutrient uplift and in so doing explores the consequences of rooting system C costs for plant P acquisition. Our approach provides useful insights for understanding plants' capacity to successfully adjust to changing soil resources in the Anthropocene.

Methods

Plant growth and soil and plant sampling

To examine the plasticity of rooting system strategies for P acquisition from organic- and mineral-bound sources, we conducted a greenhouse study in which we grew *Silphium integrifolium* (Rosinweed) seedlings (The Land Institute, Salina, KS) in inert media containing varied percentages of Po and Pi (Fig. 2).

We conducted the experiment at the University of Kansas Field Station greenhouse from June to November 2020. Greenhouse temperatures ranged from 1.67°C to 32.2°C over the growing season. Seedlings were arranged in four randomized blocks within the greenhouse. We planted seedlings in media composed of perlite amended with organically-bound P (Po) and mineral-bound P (Pi) such that the P source was either 100% Po, 100% Pi, or a depth-dependent combination (Fig. 2). Pi was sourced from research-grade apatite (Sigma-Aldrich 900195), the dominant primary mineral from which P is derived in natural systems. Roots and their mycorrhize have been observed tracking apatite and acquiring its P (Leake et al. 2008). Po was sourced from dried and finely ground cow manure, which has a ^{13}C signature distinct from that of C3 vegetation (i.e. ~ -22‰ compared to C3 vegetation signatures of that average ~-27‰), due to the corn diet of the cows. This signature permitted us to track root-derived C as it leached into the experimental growth media. P of each form was applied so that all pots contained ~0.17 mg P cm^{-3}, an application rate based on naturally occurring soil P concentrations in relatively P rich soil systems (Holleran 2013). We distributed these media into 1 m tall pots such that there were five replicates for each of the following treatments at each sampling timepoint: 100% Pi, 100% Po, 33%/66% Pi/Po, 33%/66% Po/Pi. In pots containing both Pi and Po, the materials were distributed with the lower 66% of the pot containing either Pi or Po only (~1200 mg P) and the upper third of the pot containing the alternative P form (~600 mg P). By exposing plants to treatments with Po and Pi at different depths, we were able to explore the relative effects of soil depth vs. nutrient source on plant rooting system strategy, and thus the plasticity of rooting systems as they access different P forms and redistribute P from multiple depths into their above- and belowground biomass.

Prior to the start of the experiment, five, approximately three-month-old seedlings were uprooted, washed and photographed. These untreated seedlings were then dried at 60°C for the analyses described below. The remaining rosinweed seedlings were gently washed of soil and sown into the potting media described above in early June, 2020. The potted seedlings were randomly divided into four blocks in the greenhouse to account for differences in sunlight angle and temperature in different areas of the room. Seedlings were grown for 6 months, mimicking the approximate length of the growing season to which they would be exposed naturally. At the beginning of the growing season, seedlings were watered ~100 mL tap water every day until fully established. As they grew, this changed to ~220 mL tap water 3 days per week. On two days each week, water was substituted with a liquid, P-free Hoagland's solution that was rich in all necessary plant nutrients (modified from Hoagland and Arnon 1950) except P.

Subsets of seedlings were sampled at two timepoints during the growing season. The first sampling was conducted at the end of August and the second at the beginning of November. During each sampling, potted seedlings were laid flat on a table and one side of the pot was removed with a knife to expose the root profile. Roots were then gently loosened from the profile so that aggregates attached to roots remained, and roots along with their aggregates were lifted with the roots out of the pot. We set the roots next to the pot and took photos along the length of the soil and soil profile. To quantify the depths from which soil chemical properties would be analyzed, we divided the profile into 6 depths. In the 100% Po and 100% Pi treatments, these 6 depths were distributed evenly across the profile. In the 33%/66% Po/Pi and 33%/66% Pi/Po treatments, soils were divided at 2 cm from the top of the soil, halfway between the top and upper bound of the lower soil horizon, 2 cm above the lower horizon, 2 cm below the lower horizon, the midpoint of the lower horizon and bottom of the pot, and 2 cm from the bottom of the pot. Depth integrated soils were then collected from these intervals using the denoted depths as the midpoint. These are considered "near rhizosphere" soil samples because they were often rooted but did not come out of the pot attached to the roots. Any soil clinging to roots was collected in separate bags at these same depths and labeled as "rhizosphere" soil. After collecting the soils, we cleaned the roots, took another photo of the clean root system, and then collected roots and leaves for analyses described below. Rooting systems growing in 33%/66% Po/Pi and

33%/66% Pi/Po treatments were split into either Pi-grown or Po-grown roots during collection. All samples were taken to the laboratory and frozen at -20°C until further analyses.

Lab analyses

Plant C allocation

We assessed plant C investment in belowground strategies for nutrient acquisition via several approaches. We examined rooting system exudate C found in soils as well as root abundances, as roots themselves represent a pool of plant C (Rasse et al. 2005). For soil analyses, we analyzed rhizosphere soils where available. When there was an insufficient quantity of rhizosphere soil, we analyzed the 'near rhizosphere' soil samples, designated as described above.

a. Root Abundance

We used photos taken during the sampling events to quantify rooting abundance across the depth of each pot. Photos of each uprooted seedling and remaining 'near rhizosphere' soil were downloaded and opened in ImageJ (Rasband 2018) software. Using the meter stick captured in each photograph, we set the scale in each photograph so that a known number of pixels was equated to 1 cm. We overlayed a 1 cm x 1 cm grid on the photo and generated a spreadsheet in Microsoft Excel in which one cell represented one grid cell for a 20cm wide window of the uprooted plant and a 20-30cm wide window of the remaining soil. In each Excel cell, we noted the presence or absence of a root in each corresponding photo cell. Fine (<1 mm diameter) and coarse (≥ 1 mm diameter) roots were noted separately; if a cell contained no root, the corresponding excel cell was marked 0. Presence or absence of root classes were then summed to determine the proportion of each 1cm layer that was rooted. We also summed the total roots in each pot and divided the roots in each 1cm layer by the total number of rooted cells as a way to quantify the fraction of the total roots in each soil layer. We plotted root distributions from each 1cm layer across the depth of the ~1 m potted profile for each pot.

b. Rooting system exudates

1. Extractable organic carbon

We analyzed extractable organic carbon (EOC) of soils collected from each of the 6 sampling depths in each pot as a proxy for certain rooting system exudates. EOC is known to be partly composed of organic acids, an exudate of roots, mycorrhizae, and many free-living soil microbes that reside preferentially in the rhizosphere (Herbert and Bertsch 1995) that contributes to the weathering of nutrient-containing mineral materials. To analyze EOC, we first extracted soils using 0.5 M K_2SO_4 in a ratio of 1 part soil to 5 parts K_2SO_4 solution. We shook soil slurries for 30 min at 243 rpm on a platform shaker, let the solutions settle, and then filtered them with 0.45 uM syringe filters. The extracted solutions were then pipetted into 96 well plates, mixed with sodium pyrophosphate and sulfuric acid reagents according to Bartlet and Ross (1988) and incubated at room temperature for 18 hr. After incubation, plates were run on a Bio Tek SynergyHT plate reader (VT, USA) at an absorbance of 495 nm to determine the concentration of EOC in each solution. Using the known amount of soil and K_2SO_4 that went into each solution, we calculated the EOC in each soil sample in ug C g soil^{-1}.

2. Acid Phosphatase activity

To quantify efforts by whole rooting systems to obtain organically-bound P, we analyzed soil acid phosphatase activity following the protocol outlined in Min et al. (2014). Although phosphatase can be produced by roots, their mycorrhizae, or free-living microbes (Jkujins and Burns 1976; Tarafdar and Claassen 1988; Nannipieri et al. 2011), the relative differences in phosphatase between samples serve as a metric that highlights the differences in potential P release that could be available to growing seedlings in each treatment. Briefly, we measured 1 g of each soil sample into a specimen cup and added 125 mL of 50 mM sodium acetate buffer, adjusted to approximately the same pH as the soil sample. We blended each soil slurry for 30 sec before pipetting it into a black 96 well plate, into which fluorescently-labeled acid phosphatase substrate had already been pipetted into specific columns. Plate columns contained different combinations of the fluorescent methylumbelliferyl tag itself, buffer, soil slurry, and substrate to serve as controls for background activity in each solution. We incubated the prepared plates for 18 h at room temperature, after which we ran them on a Bio Tek SynergyHT plate reader (VT, USA) set to an excitation wavelength of 360/40 and emission wavelength of 460/40. Activity was recorded in n mol P g soil^{-1} hr^{-1}.

3. Soil, root and shoot C and d13C

To discern the amount of total C in each part of the planted system—soils, roots and leaves—and to try to discern the quantity of C from roots vs. manure by the end of the experiment, we quantified the %C and the ^{13}C signature of the roots, leaves and surrounding soil media collected from each plant. To analyze soils and roots for C and ^{13}C, we dried both roots and soils at 60°C for 3-5 days until no mass loss was measured and ground them to a fine powder with a mortar and pestle before passing through a 2 mm sieve. All samples were packed into tin capsules, 2 mg for leaves, 3 mg for roots, and 8.5 mg for soils. Capsules were then sent to the Kansas State University Stable Isotope Mass Spec Lab for elemental analyzer isotope ratio mass spectrometry analyses (Elementar EA Vario Pyrocube and IRMS GeoVisION).

P stocks and uptake

a. Soil P pools

To determine the P resources available to growing seedlings over the course of the experiment, we analyzed total P, Pi and Po of soil samples collected across the depth of each pot. For pots in which rhizosphere soils were available, we focused our analyses on the rhizoshere subsamples. When there was an insufficient quantity of rhizosphere soil, we analyzed the 'near rhizosphere' soil samples.

For total P analyses, soils were dried at 60°C and finely ground using a mortar and pestle before being passed through a 2 mm sieve. Once prepared, the samples were sent to the Kansas State Soil Testing Lab for total P analysis via inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian 720-ES, Palo Alto, USA).

To analyze Po and Pi fractions, we performed the first two extractions of the Hedley extraction sequence, which extract most of the Po from a soil sample, permitting Pi and Po to be parsed. We followed the method of Tiessen and Moir (1993), as modified in Hauser et al. (2020). Briefly, we weighed 0.5 g of each dried soil out into a labeled 50 mL tube,mixed them with 30 mL 0.5 M $NaHCO_3$, and shook them overnight at 120 rpm. Samples were then removed and centrifuged for 10 min (3400 rpm, 4°C). Supernatant from the settled samples was filtered through a 0.45 uM Millipore cellulose acetate/nitrate syringe filter into a clean, labeled 50 mL tube and refrigerated. Remaining soil was mixed with 0.1 M NaOH and placed back on the shaker table to shake overnight at 120 rpm. The next day, the NaOH solutions were centrifuged for 10 min (3400 rpm, 4°C). Solutions were filtered using 0.45 um syringe filters and stored in the fridge. Prior to P analyses, each sample was acidified using 0.9 M sulfuric acid to precipitate unwanted organic matter that can interfere with colorimetric analyses.

We analyzed both solutions for inorganic orthophosphate on the biotech microplate reader (specs) in the lab at the University of Kansas using the method of D'Angelo et al. (2001).

Solutions were pipetted into 96 well plates and incubated with a malachite green reagent before plate reader analysis at a wavelength of 630 nm. The procedure generated measurements of ug Pi g soil^{-1}.

The remaining solution was shipped to the Kansas State University Soil testing lab for total P analyses via ICP-OES. Analyses returned total P ml^{-1} solution, which we converted to total P g soil $^{-1}$. We then used the following equations to calculate total Pi and total Po in each soil sample:

$$P_{\text{total, ICP (NaOH or NaHCO3)}} - P_{\text{i-Colorimetric(NaOH or NaHCO3)}} = P_{\text{o (NaOH or NaHCO3)}} \qquad \text{(Eqn. 1)}$$

$$P_{\text{O-NaHCO3}} + P_{\text{O-NaOH}} = P_{\text{O, extract total}} \qquad \text{(Eqn. 2)}$$

$$P_{\text{total soil, ICP}} - P_{\text{O, extract total}} = P_{\text{I, total}} \qquad \text{(Eqn. 3)}$$

Each P pool was recorded in mg P g soil $^{-1}$ and compared across treatments, soil depths and time points.

b. Plant biomass P pools

We analyzed the P found in the harvested leaves and roots from each seedling to determine the P contents in the aboveground and belowground plant components. These components approximate the amount of P taken up by the plant over the course of the experiment. To analyze leaf and root P, we dried the harvested biomass at 60°C, ground each component with a mortar and pestle to pass through a 2 mm sieve and sent them to the Kansas State Soils Testing Lab for total P analysis using ICP-OES.

Statistical Analyses

Changes in C and P stocks over the course of the experiment demonstrate ways in which rooting systems respond to and redistribute resources over time. To analyze changes to C and P stocks, we applied a mixed effects ANOVA on leaf, root and soil P stocks with soil treatment and timepoint as fixed effects and sampling block as a random effect. Soil P stocks and root P stocks were each summed across the depth of the pot so that each response, soil P or root P, represents the total P from each pool in a given sample pot. Depths were not considered separately because we wanted to compare the net change to each P pool over the course of one growing season. Changes to soil P stocks relative to root and shoot P stocks over time were used to investigate nutrient uplift. With one exception, data met the model assumptions of normal residuals, homogeneity of variances, and independent samples. Soil Po stocks required log transformation to meet these assumptions.

Root distributions and the distribution of acid phosphatase across treatments, soil depth and sampling time were analyzed using a mixed effects linear model to discern the degree of plant response to distinct P pools. We ran one model to analyze the response of root fraction (including, separately, the proportion of each 1 cm row inhabited by roots and the fraction of total roots) as a function of treatment, timepoint and depth, with sampling block as a random effect. We ran a second model to analyze acid phosphatase activity as a function of treatment, timepoint and depth, also accounting for the random effect of sampling block. Both rooting depth and phosphatase data were log transformed to meet the assumptions of normal residuals, homogeneity of variances, and independent samples. The model generated for the fraction of fine roots per row failed to meet the assumption of homoskedasticity, even after data transformation;

this relationship thus was investigated using non-parametric quantile regression to analyze the median of the data.

Finally, we looked for relationships between plant nutrient uptake mechanisms and soil P pools as a measure of plant response to distinct nutrient forms. To do this, we ran a correlation analysis between the two root abundance metrics described above, which we transformed from 1cm rows into depth integrated blocks to match the soil sampling depths from each pot, and soil P forms, focusing on Po and Pi stocks. Note that our primary object of interest for these analyses was whether change in root abundance was correlated with change in soil P, and not the effect of depth on either variable. We looked for evidence of nutrient uplift in these data by examining correlations between changes to root abundance and changes to soil P pools. Data met the assumptions of normal residuals, homogeneity of variances, and independent samples, except in the case of fine root fraction per 1 cm horizon, which had to be log transformed to meet model assumptions. All data were analyzed in Rstudio v. 1.2.5033 using the lme4 and quantreg packages (Bates et al. 2015, studio Team 2019, Koenker 2021).

Results

P Stocks

We observed significant differences in P stocks in soils, roots and leaves between treatments and between sampling timepoints. Both leaf and root P stocks showed significant differences in P between soil treatments (leaves: F = 4.39; P = 0.011; roots: F = 3.14; P = 0.04), with larger P stocks in pots containing Po (Fig. 3). Root and leaf P increased over time across treatments (leaves: F = 7.88; P = 0.008; roots: F = 12.38; P = 0.001), although there was very little change in plant biomass for seedlings grown in Pi-only soil.

Soil P stocks also varied between treatments and timepoints (Fig. 3). Po stocks differed between treatments ($F = 377.05$; $P < 0.00001$) and changed significantly over time ($F = 20.65$; $P = 8.26e^{-5}$) with declines in all treatments. Soil Pi stocks did not change significantly over time but were different between treatments ($F = 41.12$; $P = 4.13e^{-11}$).

Rooting system C allocation mechanisms

Two of our root abundance metrics, fraction of total roots per 1cm row and fraction of fine roots per 1 cm row, demonstrated a significant effect of the interaction of depth, soil treatment and time (total roots: $F = 16.3$; $P = 1.53e^{-10}$; fine roots: $F = 10.01$; $P = 1.42e^{-6}$). Root abundance, both total and fine, increased over time particularly strongly in pots containing both Pi and Po. The increase in root abundance over time appeared greatest at depth in these two treatments (Fig. 4).

Soil phosphatase activity demonstrated a significant effect of the interaction of soil depth and treatment ($F = 51.13$; $P < 0.00001$). Phosphatase activity increased with depth in the PiPo treatments, such that phosphatase activity in deep soil was comparable to that in Po only pots, and decreased with depth in the PoPi treatments, such that phosphatase activity in deep soil was comparable to that in Pi only pots (Fig. 4).

Relationships between rooting system responses and soil P pools

We observed correlations between changes to root abundances over time and changes to P stocks over time that point toward relationships between plant C allocation to root growth and soil P. In pots with Pi layered over Po, we observed a significant negative correlation between the change in total root fraction per 1 cm row over time and the change in soil Po over this same timeframe (Fig. 6, adjusted $r^2 = 0.71$; $P = 0.02$). We observed a similar relationship for changes to fine roots per 1 cm row (adjusted $r^2 = 0.672$; $P = 0.029$).

In pots with Po layered over Pi, we observed a significant positive correlation between the difference in fraction of total roots per 1 cm row over time and the change in soil Po over time (Fig. 6, adjusted $r^2 = 0.55$; $P = 0.056$), as well as a positive correlation between the change in fraction of fine roots per 1 cm row over time and the change in soil Po (adjusted $r^2 = 0.68$; $P = 0.03$). Additionally, when examined as a fraction of the total number of rooted cells, the change in total roots over time showed a significant positive correlation with the change in soil Po (adjusted $r^2 = 0.81$; $P = 0.009$). The change in fine roots as a fraction of the total number of fine roots showed a similar positive correlation (adjusted $r^2 = 0.85$; $P = 0.006$).

Discussion

Our findings provide evidence that plants exhibit efficient and plastic exchanges of C-for-P that reflect the availability of distinct P forms over time, regardless of the depth at which those P forms are distributed in the soil profile (Fig. 1). In natural systems, organic nutrient sources are typically found in shallow soil horizons (Jackson et al. 2000; Jobbagy and Jackson 2001; Richter et al. 2006; Marschner and Rengel 2007); thus, placing organic P in deep soils allowed us to test the extent to which seedlings growing in those inverted soil profiles could exhibit plasticity in their strategies for P acquisition. However, regardless of the depth distribution of the organic P source, we observed consistent, preferential plant C allocation to rooting system proliferation and acid phosphatase enzymes at depths where organically-bound P was abundant, in conjunction with evidence of successful plant uptake of P from those organic sources. These results suggest that organically-bound P is a readily available plant P source. Additionally, our observations of plants allocating C to strategies capable of leveraging organically-bound P, even when that P was distributed in deep soils, provides evidence for plant reliance on nutrient uplift when bioavailable P could be obtained from deep soils. Our study thus demonstrates some of the first direct observations of nutrient uplift, as well as evidence that rooting systems are plastic in their response to soil nutrient resources.

Plants demonstrate plastic P uptake strategies across depth to acquire organically-bound P

Changes to soil P stocks over the course of the experiment demonstrate ways that plants redistributed P in our experimental system. The decline in organic P stocks over the course of the experiment in all treatments and the relatively smaller change to mineral-bound P stocks suggest that organically-bound P served as a readily available source of plant P compared to mineral-bound P in these soils (Fig. 3). These changes in soil P occurred alongside increases in plant P in roots and shoots (Fig. 3), suggesting that plants were likely drawing on these organic P pools for construction of new plant material. While soluble inorganic P is often emphasized in studies of plant P sources (Hinsinger 2001; Achat et al. 2016), numerous lines of evidence suggest that plants adopt multiple strategies to leverage the combinations of organic and inorganic P that are available to them, and that organically-bound P represents a key component of plant P nutrition (Paungfoo-Lonhienne et al. 2012; Andrino et al. 2019; Lugli et al. 2019; Hauser et al. *in review*). Our experiment provides further support for the idea that organic P is indeed a meaningful source of plant P. Given high levels of plant productivity even in highly weathered, mineral-P depleted soils (Yang and Post 2011; Cleveland et al. 2013), we would anticipate that organic P can be a readily accessible and valuable source of P for plants across systems.

Soil treatments in which organic P was layered over mineral-bound P serve as an important test of the plasticity of plant P uptake strategies. Typically plant uptake of organically-bound P would dominate plant nutrient acquisition in shallow soils because that is where the bulk or organic P resides (Richter et al. 2006). Thus, to access deep, organically-bound P sources in the inverted soil profiles represented by layering mineral P over organic P requires that roots perform P uptake processes characteristic to shallow soils in relatively deep soils instead. Indeed, seedlings in soils with organic P layered underneath mineral-bound P exhibited evidence of obtaining their P from the more deeply distributed organic P, given that deep organic P stocks declined over the course of the experiment and shallower, mineral-bound P stocks exhibited no change over time (Fig. 3). These observations suggest that organic P was preferentially utilized as a P source for growth, and that plants switched to this more readily available nutrient source once roots proliferated deeply in the soil profile.

Whole-rooting system C allocation changes across depth to enhance access to bioavailable P

Seedlings allocated C to root growth in patterns that would suggest that plants economize C to access bioavailable, organically-bound P. One of the primary ways that plants allocate C to nutrient acquisition is by construction and proliferation of new roots (Nadelhoffer and Raich 1992; Kumar et al. 2018; Stock et al. 2021), which represent a substantial C investment (Nadelhoffer and Reich 1992). Rooting systems in seedlings in the current study proliferated as extensively in deeply distributed organic P pools as they did in shallowly distributed organic P pools (Fig.4), suggesting that plants are capable of adapting their C allocation to root growth at any depth that permits access to the most readily bioavailable nutrient form. Proliferation of deep roots where organic P was layered underneath mineral-bound P (Fig. 4) especially highlights that plants' C allocation to roots is sufficiently flexible to leverage this P pool regardless of its depth in the soil profile. Roots growing in soils with organic P layered over mineral P exhibited the greatest increase in overall root abundance at depth over the course of the experiment (Fig 4), but this is likely a result of the advantage these seedlings gained from encountering shallow, readily available Po upon planting that the root systems planted in mineral P layered over organic P did not. These root growth pattens are consistent with those observed in the field in similar nutrient environments (Hauser et al. 2020) but observing this phenomenon in a controlled setting highlights the capacity plants have to change C allocation across soil depth in response to nutrient forms.

Alongside root proliferation, another plant C allocation pattern that points toward plants' capacity for plasticity in nutrient acquisition strategies is the distribution of extracellular enzymes across soil depth. Hydrolytic extracellular enzymes, such as acid phosphatase, cleave nutrients from organic molecules, making them especially relevant to the bioavailability of organically-bound nutrients such as P (Bunemann and Condron 2007; Bunemann et al. 2011). We observed shifts in acid phosphatase production in our soil treatments across depth that paralleled the changes to soil organic P, suggesting that rooting systems may be leveraging this C allocation strategy to obtain P from organic matter in addition to root production (Fig. 5). Acid phosphatase production increased in soils containing organically-bound P, pointing toward C allocated to accessing this P pool, even where it was distributed deeply in the soil profile.

Phosphatase production as a measure of rooting system C economies must be examined while being mindful of two important caveats. First, phosphatase is a C-rich compound, thus requiring significant C investment for its production (Bunemann et al. 2011; Andrino et al. 2019). As a result, phosphatase represents a seemingly inefficient use of plant C if there are other, less C-expensive mechanisms for plant P acquisition. Additionally, phosphatase is produced by multiple actors in the soil system: roots; root mycorrhizal symbionts; and free-living microbes (Jkujins and Burns 1976; Tarafdar and Claassen 1988; Nannipieri et al. 2011). This feature makes it challenging to pinpoint the organism in the soil system responsible for the C that went into producing the observed enzyme activity. However, the production of acid phosphatase by soil microbes releases organically-bound P into soils that plants can also access (Richardson and Simpson 2011). In such a scenario, plants do not need to produce their own phosphatase to liberate organically-bound P, a phenomenon that reduces the plant's C cost of obtaining organic P and instead can transform this P source into an economically efficient C-for-P exchange from the plant perspective (van der Heijden et al. 2008, Harvey et al. 2009). The relatively high acid phosphatase activity in any organic P containing soils in our experiment occurred alongside higher root abundances in those same soils (Figs. 4 & 5), suggesting that regardless of whether phosphatase emanated from roots or microbes, these rooting systems were poised to access any P that was released. Thus, in spite of the emphasis in the literature on mineral-bound P as a key P resource for plants in a diversity of ecosystems (Lambers et al. 2008; Leake et al. 2008; Burghlea et al. 2015; Dontsova et al. 2020; Hauser et al. *in review*) the current study highlights how roots may take advantage of P released by phosphatase generated by roots or microbes, and that microbially-generated phosphatase can reduce the amount of C plants themselves have to allocate to an otherwise expensive P acquisition mechanism.

Plasticity in rooting system C-for-P strategies produces observable nutrient uplift with implications for development of Earth's CZ.

Whether driven by the roots themselves or their microbial neighbors, organically-bound P appears to have served a greater P benefit than mineral-bound P to growing seedlings, so much so that we observed evidence of nutrient uplift from organic P stocks where they were distributed deeply (Fig. 6). We observed changes to root abundances relative to changes in P concentrations in deep soils that suggest that roots were successful at uplifting deeply distributed P. First, in soils with mineral P layered over organic P, root and shoot P stocks increased over time while soil organic P stocks declined, and inorganic P stocks did not (Fig. 3). This implies that rooting systems in these layered treatments must have obtained their P by uplifting deeply distributed organic P. Additionally, in soils with mineral P layered over organic P, we observed that the greatest increase in roots, especially in deep soils, correlated with a decrease in organic P (Fig. 6), suggesting that in deep soils where organic P was initially abundant, root proliferation was a successful mechanism of plant P uptake and redistribution. On the other hand, roots also increased in abundance in deep soils where organic P was layered over mineral-bound P, but the small amount of deep organic P in these soils did not change (Fig. 6). This finding suggests that rooting systems likely had either gotten sufficient P from shallow organic P or were tapping into deep mineral-bound P for nutrition. Given the decline in shallow organically-bound P in these pots (Fig. 6), the former is more likely, again pointing toward the utility of organic P as a nutrient source. Roots were abundant in these shallow, organic P-rich soils throughout the experiment, suggesting that plants had access to readily available organic P, even at the first sampling timepoint, and therefore did not perform nutrient uplift where organic P was shallowly distributed. These relationships between root proliferation and Po depletion demonstrate that where the preferential P form is deeply distributed, plants will allocate C to deeper root systems to obtain it, which will induce nutrient uplift.

The process of nutrient uplift is important for redistributing bioavailable nutrients across Earth’s CZ (Jobbagy and Jackson 2001; Dontsova et al. 2020). Here, we demonstrate that nutrient form is an important driver of plant nutrient uplift given differential plant responses to deeply distributed organically-bound P and mineral-bound P. In light of anticipated changes to future soil nutrient stocks (Amundson and Jenny 1991, Haff 2010, Yoo and others 2015) and to rooting depths themselves (Hauser et al. *in review*), these findings imply that nutrient uplift patterns could also change in the future. Thus, the C-for-P economies of plant rooting systems, and the capacity of those economies to change, will be important for predicting the developmental trajectory of Earth's CZ.

Conclusion

Our findings demonstrate mechanisms by which plants shape Earth's soil C and nutrient distributions, as well as reveal ways plants may cope with future changes to soil resource availability. Through a greenhouse experiment controlling for the depth distribution of P forms, we provide evidence that plants exhibit plasticity in their nutrient acquisition strategies where such plasticity produces economical C-for-P exchanges. In our study system, organically-bound P appeared to be preferentially used by plants to a greater extent than mineral-bound P, in spite of the greater C cost of extracellular enzyme production relative to the organic acids that are typically thought to be useful for release of mineral-bound P. This observation may emerge as a consequence of microbial activity in and around plants' rooting systems. The release of P from organically-bound forms, whether via activities of roots themselves or their microbial neighbors, provided enough incentive for roots to proliferate deeply and perform directly observable nutrient uplift. This suggests that plants adopt nutrient uptake strategies targeted at the most efficient C-for-P exchanges, shifting their strategies across soil depth to leverage the most bioavailable nutrients. Rooting systems, therefore, are plastic in their responses to their soil nutrient environments, an important characteristic considering anticipated shifts in soil nutrients and root distributions in the years to come. Our study suggests that, given such changing environments, rooting systems are capable of adapting their strategies for nutrient acquisition to acquire sufficient nutrition by promoting processes like nutrient uplift that redistribute soil resources. In responding to and redistributing distinct nutrient stocks, rooting systems also influence the fate of C belowground, implicating nutrient acquisition as a driver of patterns of belowground C cycling as nutrient sources vary across space and time. Therefore, rooting systems' strategies will play a meaningful role in the ability of Earth's critical zone to maintain carbon and nutrient cycling functions in the presence of global anthropogenic change.

Figures

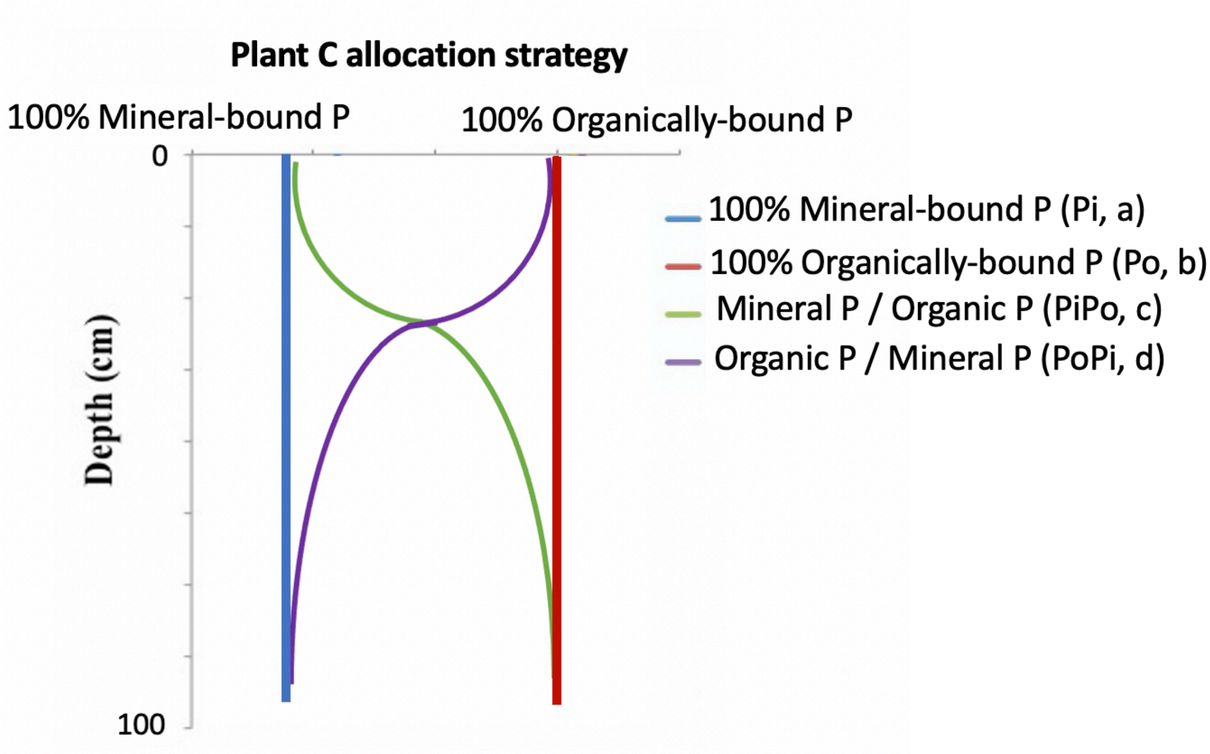

Figure 1. Generalized diagram of experimental hypotheses. We hypothesize that plant rooting systems will exhibit P uptake and C allocation responses (plant strategy on the X axis) specific to each P form across the depth of each pot over the course of the growing season. For example, we anticipate plant C allocation to roots grown in 100% mineral-bound P soil (a) to produce a pattern that is distinctly different from roots growing in 100% organically-bound P soil (b). In pots with mineral-bound P layered over organically-bound P (c) we anticipate that plant C allocation to rooting systems will be more similar to that in mineral-bound P soils at the surface and more similar to that in organically-bound P soils in deeper horizons. We anticipate the opposite where organically-bound P is layered over mineral-bound P (d), with rooting system C allocation most similar to that in organically-bound P soils at the surface and most similar to that in mineral-bound P soils at depth.

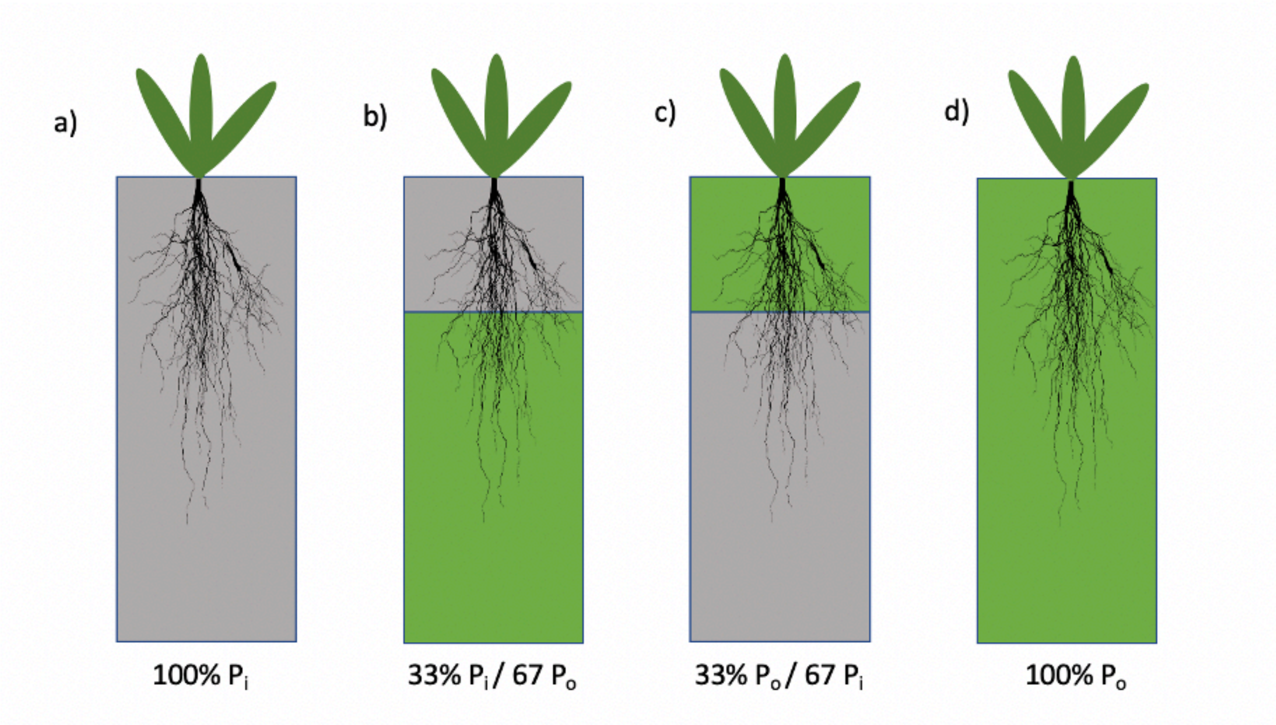

Figure 2. Depiction of experimentally manipulated P resources for greenhouse-grown seedlings exposed to varying depth distributions of of mineral-bound P (Pi) and organically-bound P (Po). Seedlings were planted in soils containing either 100% Pi (a), Pi layered over Po (b), Po layered over Pi (c), or 100% Po (d).

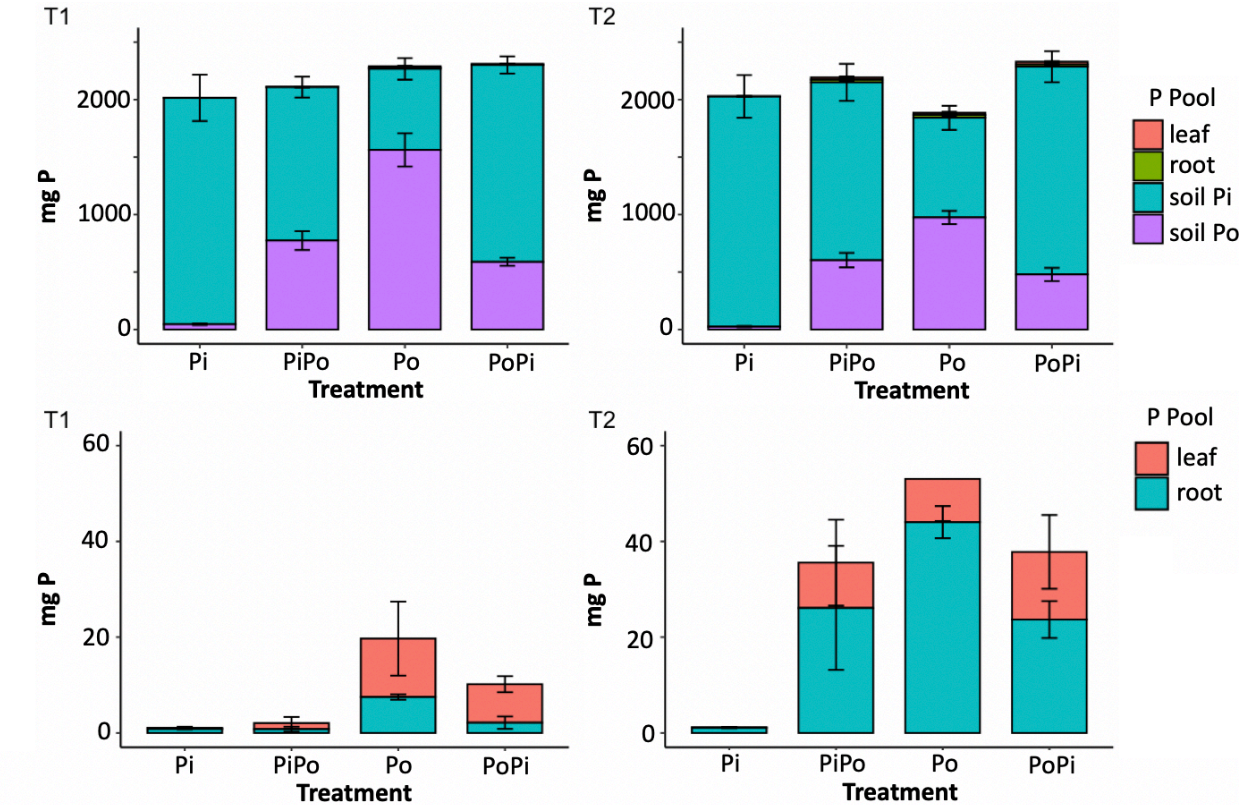

Figure 3. Summary of P stocks in mg P at the first timepoint (T1) and second timepoint (T2) of the experiment for the four soil treatments described in Fig. 2. Top two plots represent both soil and plant P stocks; lower two plots represent only leaf and root data. We observed significant increases in plant P over time (leaves: F = 7.88; P = 0.008; roots: F = 12.38; P = 0.001) and between treatments (leaves: F = 4.39; P = 0.011; roots: F = 3.14; P = 0.04). There were no significant changes to mineral-bound soil Pi stocks over the course of the experiment, but soil Po declined over time in all treatments ($\chi^2 = 18.9$; P = $1.4e^{-5}$).

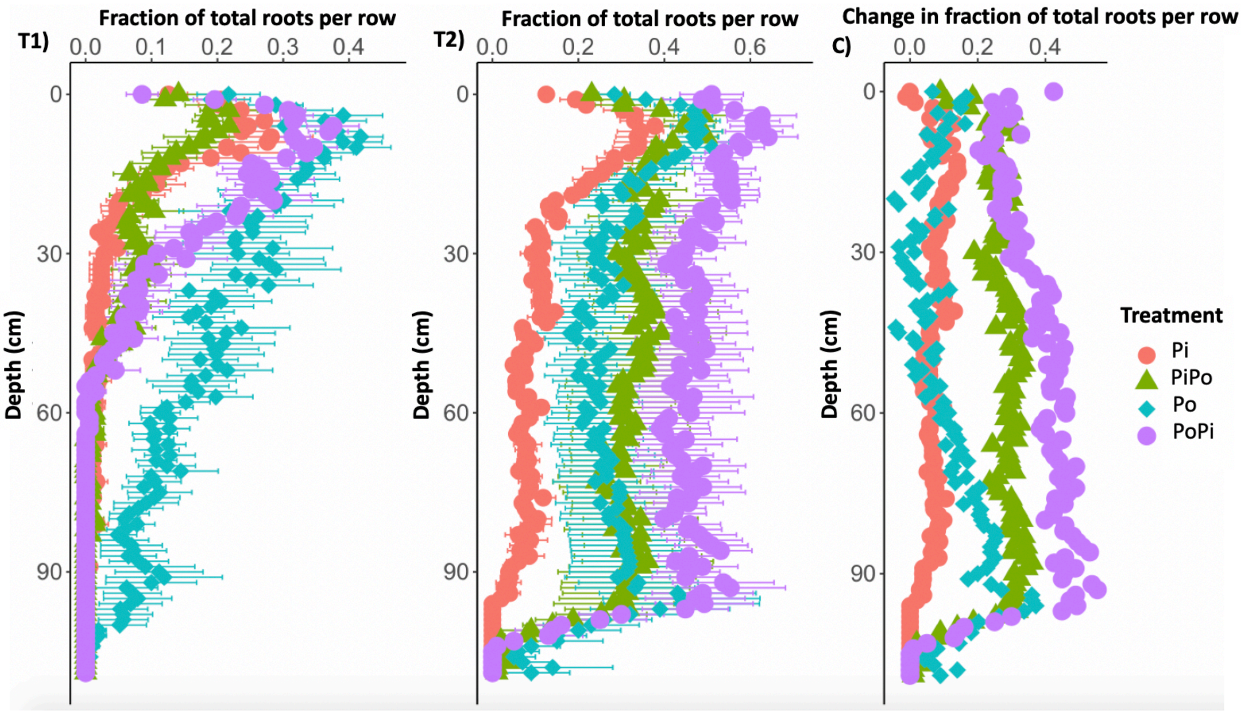

Figure 4. Fraction of each 1 cm soil depth containing roots, both coarse roots and fine roots, at timepoint 1 (T1), 5 months after planting, and timepoint 2 (T2), 5 months after planting. Changes to roots were significantly different in different treatments at different depths over time total roots: F = 16.3; P = $1.53e^{-10}$), with the greatest increases to roots in the PiPo and PoPi pots. Panel (C) shows these differences visually, with each point representing the difference of the mean fraction of roots in each treatment at each 1cm depth. Error bars represent one standard error of the mean.

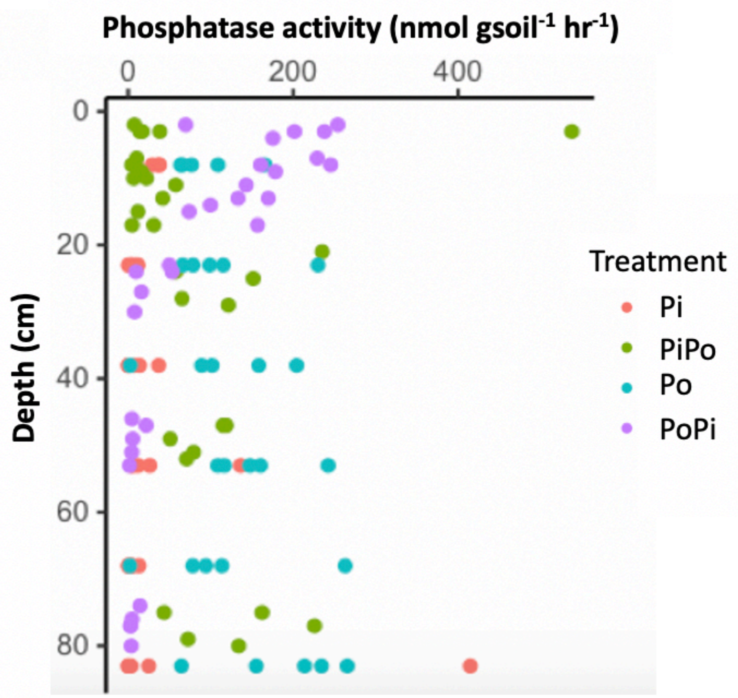

Figure 5. Phosphatase activity (nmol $hr^{-1}gSoil^{-1}$) across soil depth (cm) after six months of seedling growth. Phosphatase activity was significantly different between treatments at different depths ($F = 51.13$; $P < 0.00001$) such that there was greater activity in organic P containing soils.

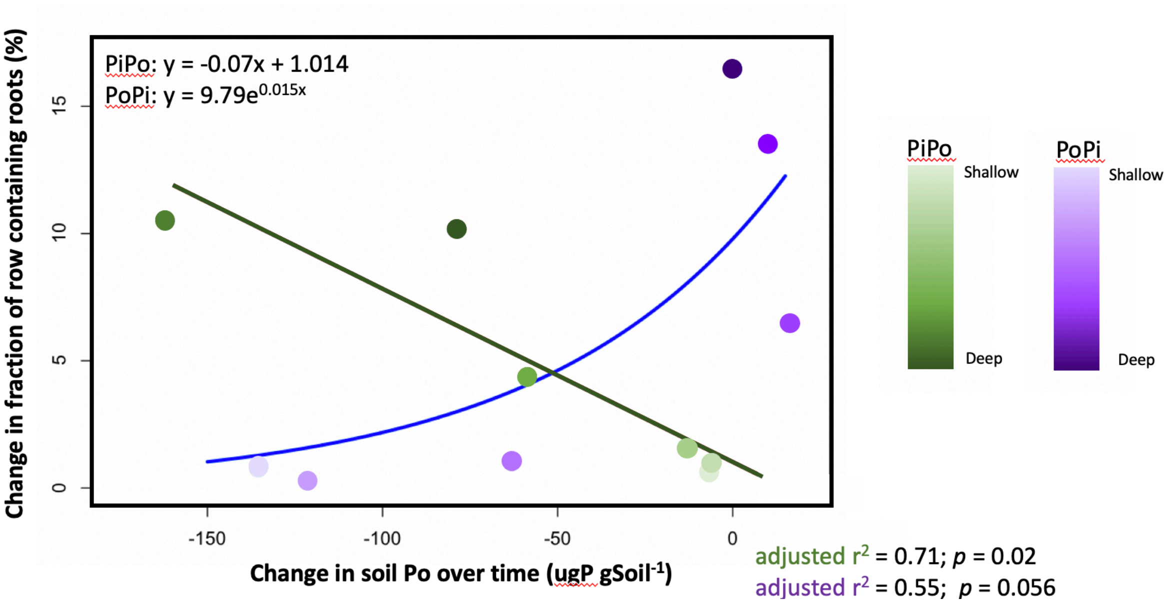

Figure 6. Change in fraction of each 1cm row containing roots compared to the change in organic phosphorous between the two experimental timepoints. There is a significant positive correlation between change in organic P and change in roots in soils where organic P is layered over mineral-bound P (blue-toned points, with darker points indicating deeper soils; adjusted $r^2 = 0.55$; $P = 0.056$) and a significant negative correlation where mineral-bound P is layered over organic P (red-toned points, with darker points indicating deeper soils, adjusted $r^2 = 0.71$; $P = 0.02$).

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General Conclusions

Understanding nutrient sources and the mechanisms by which they become bioavailable in Earth's ecosystems is critical to predicting the ways our environments will respond to agents of global change. In these studies, my goal was to explore both how and where vegetation obtains its nutrition across spatial and temporal scales. My approach is distinct from that of horticulturalists and agronomists, who have studied related processes for centuries (Leo 1963, Kurtz 1970, Comerford 2005, Chien et al. 2011). Instead, I rely heavily on observational studies from unmanaged systems and attempt to integrate biologic and geologic hypotheses to investigate vegetative responses to shifts in environmental conditions and to changes in nutrient sources that occur during the development of Earth's critical zone (CZ), as well as how those responses feedback to metrics of soil development. Thus, these studies illuminate resources and mechanisms that help sustain CZ productivity.

In chapter 1, I examined forest ecosystems' nutrient sources to discern the degree to which vegetation at the ecosystem scale draws on mineral vs. organic soil nutrient pools for productivity. I found that, while organically bound nutrients may comprise a greater proportion of the soil nutrient stock over the long timescales relevant to soil development, vegetation transitions from organic to mineral nutrient sources as roots deepen and encounter less weathered materials, even in relatively well-weathered profiles. However, in chapter 2, I reveal that the nutrient economies that develop across rooting depth are likely subject to change on a broad scale due to land use change-induced changes to rooting distributions. On average, rooting depths are becoming globally shallower as a consequence of agricultural establishment across much of Earth's terrestrial surface. During land cover transition to agriculture, deeply rooted perennial vegetation is replaced by shallowly rooted annual row crops with less access to deeper soil resources. These root shallowing effects are counteracted in some regions of the globe by woody encroachment, with introduces deeper shrub and tree roots into formerly herbaceous, and therefore more shallowly rooted, regions. This suggests that plant nutrient acquisition strategies that are driven by rooting depth may be subject to widespread change.

In chapters 3 and 4, I investigated some of the biogeochemical consequences of these changes to rooting depth, and the mechanisms by which plants respond to both changes in rooting distributions and changes to nutrient stocks. In chapter 3, I found that rooting systems develop specific belowground C allocation strategies, including production of acid phosphatase and organic acid exudates, in response to organic vs. inorganic nutrient forms. These rooting system C responses changed over soil depth such that rooting systems made efficient exchanges of C for nutrients across depth. This efficiency produced deeply rooted nutrient economies leveraging mineral-bound nutrient pools in unperturbed forests, a finding that corroborates findings from chapter 1. In post-agricultural regenerating forests, however, rooting systems' C for nutrient economies shifted toward shallowly distributed, organically-bound nutrient sources, which required a comparatively large investment in plant C toward phosphatase production and toward mycorrhizae that also produce these enzymes. These findings point toward ways that rooting systems can alter soil development, contributing to the depths and rates at which soil weathering proceeds and organic matter accumulates.

These root-mediated redistributions of mineral and organic materials have consequences for metrics of soil development, ultimately producing additional uncertainty around these metrics given the plethora of subsurface actors that can contribute to similar soil development processes. Therefore, understanding the mechanisms underlying the C and nutrient distributions we observed in the field required a manipulated experiment that would give us sufficient control to tease apart root-specific contributions to soil C and nutrient stocks. In Chapter 4, I carried out a controlled greenhouse experiment to examine root mechanisms of C allocation for nutrient acquisition. The experiment demonstrated that rooting systems have characteristic phosphatase and rooting distribution responses to organic- vs. mineral-bound nutrients and that these responses are consistent, no matter the depth of the given nutrient form. Thus, rooting systems exhibit plasticity in their rooting system nutrient acquisition mechanisms across soil depth, likely allowing them to shift strategies as soil environments change.

Together these studies point toward rooting depth as a key ecosystem trait that determines which nutrient sources vegetation will be able to access and rely on for productivity. At the ecosystem, soil pedon, and rooting system scales, I observed that rooting depth, at least in part, determined which C for nutrient exchanges plants performed. In unperturbed systems, deepening roots produced a predictable pattern of reliance on mineral bound nutrients over time, in spite of accruing organic nutrient stocks in shallower soils, which suggests that those deeply distributed resources may represent a more efficient resource exchange once roots can reach them. Evidence that the enzymes needed to release nutrients from organic molecules contain more C than organic acids that liberate nutrients from soil minerals (Hauser et al. 2020) further supports the idea that exudation patterns required to release nutrients from mineral-bound forms may be less C expensive once roots grow deeply enough to access them. Rooting depth, therefore, is critical to determining which nutrient sources vegetation relies on over time.

These studies also demonstrate that both rooting depths and the depth distributions of nutrient forms are subject to widespread changes that appear to leave a legacy in vegetations' C for nutrient economies. Rooting distributions are changing at the global scale, trending toward shallower distributions on average, which may have contributed to altered nutrient uptake patterns in the ecosystems where I saw this change occur. At the Calhoun Critical Zone Observatory, I observed shallower rooting systems in post-agricultural forests and, even after 80 years of growth, these forests maintained a nutrient economy reliant on shallowly-distributed, organically-bound nutrients, likely as one consequence of their shallower roots. Even after nearly a century, the belowground nutrient economy in these forests remains altered. In systems where both rooting distributions and the distribution of nutrient forms changed, such as at the University of Kansas Field Station (KUFS) examined as a part of chapter 1, I observed that vegetation relied unexpectedly on mineral bound nutrient forms despite its relatively young rooting system. A legacy of agricultural fertilization left shallow soils in KUFS relatively rich in mineral bound nutrients, to which rooting systems responded by utilizing those mineral-bound nutrients, making the most efficient C for P exchange. Thus, at both the ecosystem and soil pedon scales, I observed changes to plants' nutrient sources and belowground C allocation patterns that lasted for decades, likely as a consequence of land use change. However, these findings also suggest that, in spite of lasting changes to rooting depths and nutrient sources, the vegetation is able to adapt its nutritional strategies to most efficiently meet the challenges of changing soil environments.

My findings in the greenhouse support this observation-based proposition that plants are efficient and plastic economists, capable of responding to environmental change to at least some degree. In the greenhouse we gave seedlings the ultimate test of rooting system plasticity, providing them with mineral-bound P layered over organically-bound P, an arrangement of soil nutrients almost never found in natural soil horizons. Yet, plants in these soils grew and even thrived. Their rooting systems responded by proliferating at depths where nutrient forms were most bioavailable and they exhibited changes to exudation patterns emphasizing phosphatase production once they encountered deeply distributed organic P forms. Thus, rooting systems' nutrient acquisition strategies appear plastic in their response to different soil nutrient stocks. Alongside my observational studies, this finding suggests that even as soil environments change, plants will be able to efficiently adapt their economies to a variety of situations.

Thus, these studies point toward rooting depth and rooting systems processes as mechanisms by which ecosystems can respond to changes in resources over time. Such findings are important for piecing together our growing picture of how Earth's CZ develops and functions (Richter and Billings 2015), particularly as human influence rapidly alters CZ dynamics (Kumar et al. 2018). As mediators between Earth's surface and subsurface, rooting systems are capable of propagating anthropogenic changes to Earth's surface deeply through the CZ, likely with many unanticipated consequences for how Earth's CZ will function in the future (Hasenmueller et al. 2017, Billings et al. 2018). A greater understanding of how rooting systems processes change over depth and time can help us better grasp the magnitude of human influence in Earth's living skin. This understanding can improve our predictions about the future functioning of the CZ and can reveal ways for human activity on Earth's surface to support a thriving and productive CZ, even meters beneath our feet.

Introduction and Conclusion References

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Appendices

Appendix 1: Chapter 2 supplementary material

Here we include additional figures and a data table, generated via the same methods detailed in the main text. The figures represent an extension of the figures presented in text and the table represents alternate ways of calculating rooting depth metrics using different representations of rooting depths for desert biomes.

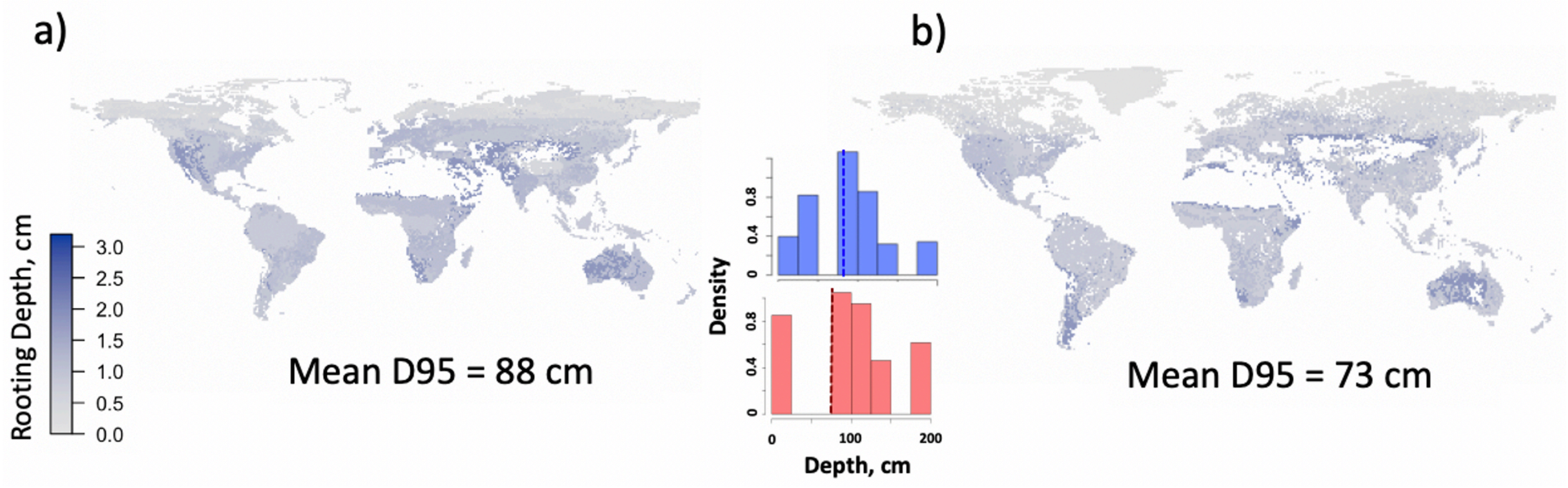

Figure S1. Comparison of potential (A) and contemporary (B) distributions of depth to 95% rooting biomass (D95) across the globe. Inset histograms display the distribution of rooting depths in each map, with dashed lines marking the means of the two datasets (blue histogram represents potential vegetation, red contemporary). The appearance of a distinct line between rooting depths at 60°N is an artifact of restricted maximum rooting depth assignments at northern latitudes used in our calculations to account for growth limitations imposed by frozen soils. Note that in both maps, Greenland root depths have a value of zero and are colored white and grey in the potential and contemporary land cover maps, respectively, due to differences in zero value representations in rasters.

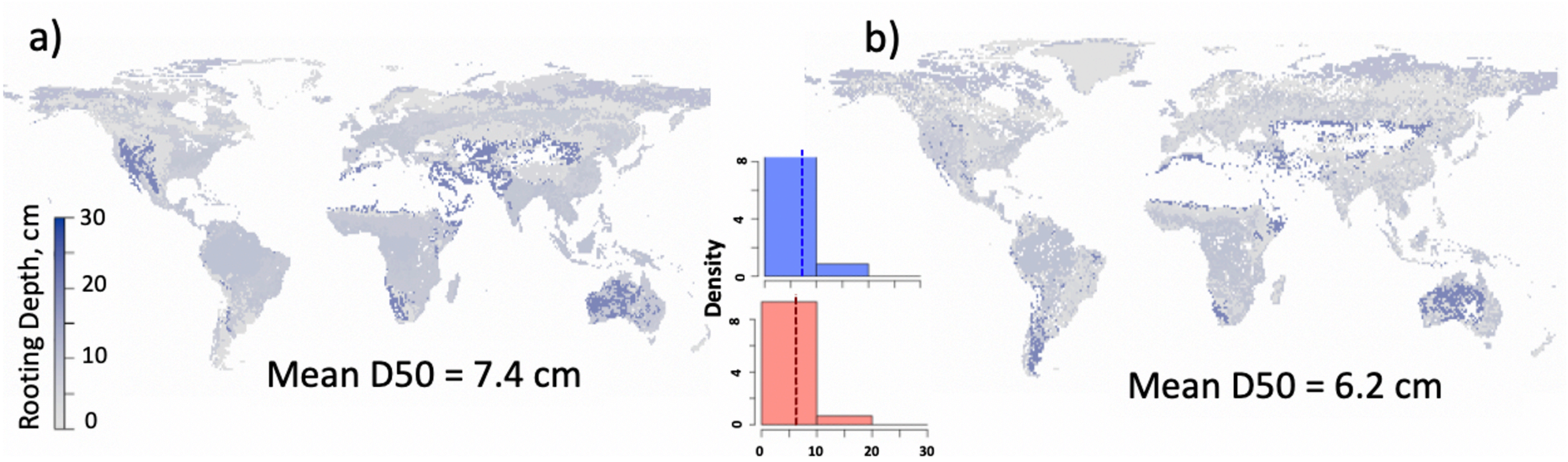

Figure S2. Comparison of potential (A) and contemporary (B) distributions of depth to 50% rooting biomass (D50) across the globe. Inset histograms display the distribution of rooting depths in each map, with dashed lines marking the means of the two datasets (blue histogram represents potential vegetation, red contemporary). The appearance of a distinct line between rooting depths at 60°N is an artifact of restricted maximum rooting depth assignments at northern latitudes used in our calculations to account for growth limitations imposed by frozen soils. Note that in both maps, Greenland root depths have a value of zero and are colored white and grey in the potential and contemporary land cover maps, respectively, due to differences in zero value representations in rasters.

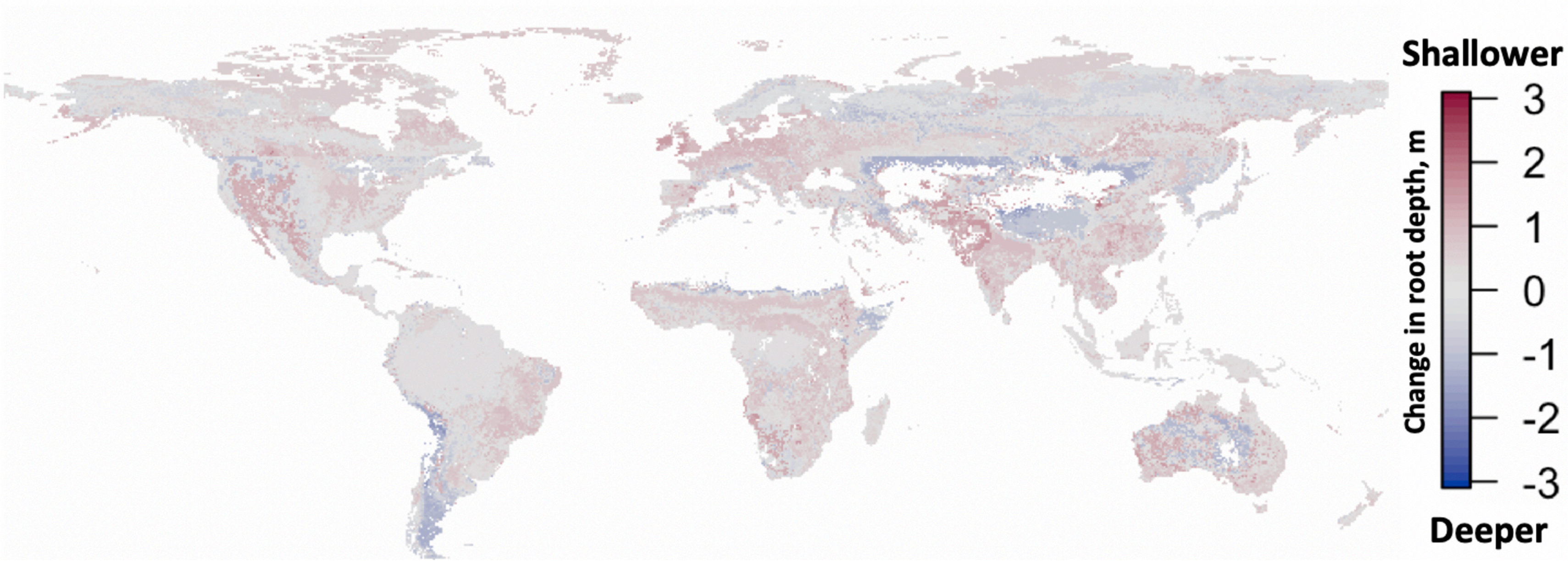

Figure S3. Change in depth to 95% rooting biomass (D95) due to differences in potential vegetation distributions compared to contemporary vegetation distributions. Red regions denote shallower roots in contemporary systems, while blue regions denote deeper roots in contemporary systems when compared to their potential vegetation distributions.

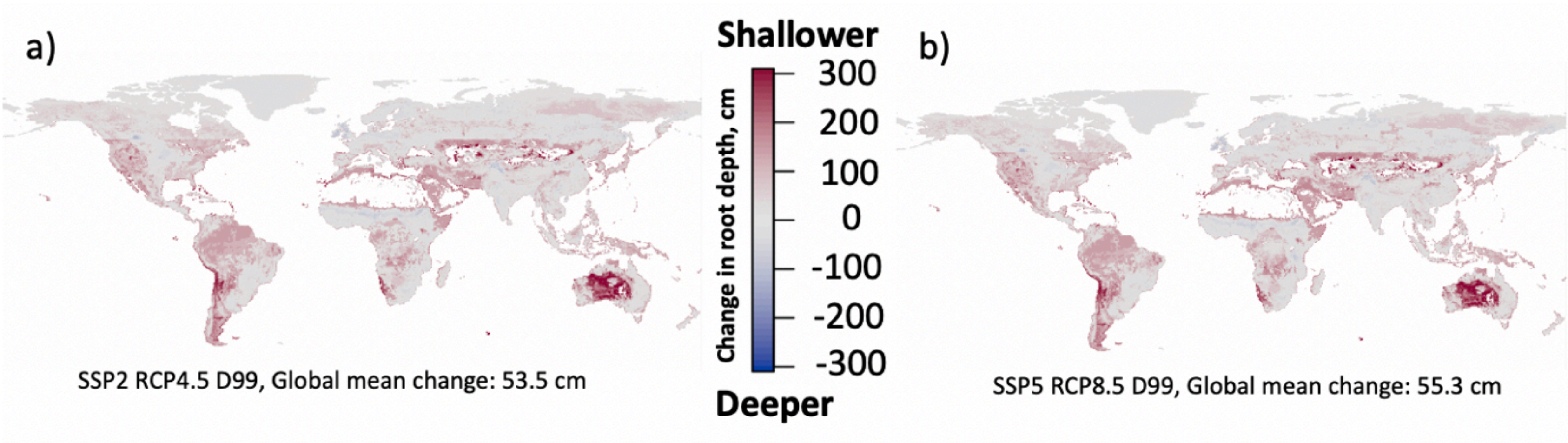

Figure S4. Change in depth to 99% rooting biomass (D99) due to differences between contemporary rooting depth distributions and anticipated rooting distributions under two projected SSP RCP scenarios for the year 2100; SSP2 RCP4.5 (a) and SSP5 RCP8.5 (b). Grey and red colors indicate root depth truncation and blue indicates elongation.

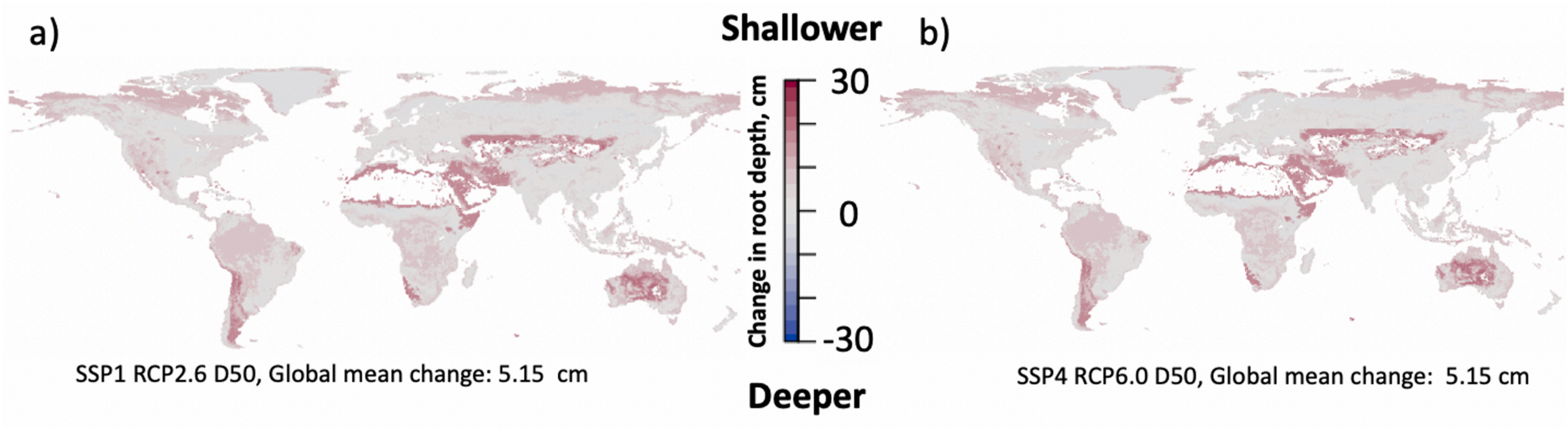

Figure S5. Change in depth to 50% rooting biomass (D50) due to differences between contemporary rooting depth distributions and anticipated rooting distributions under two projected SSP RCP scenarios for the year 2100 (results are similar for both scenarios). Projections are based on land use and emissions changes under two combinations of Shared Socioeconomic Pathways (SSP) and Representative Concentration Pathways (RCP), SSP1 RCP2.6 (a) and SSP4 RCP6.0 (b). These two maps represent scenarios of greatest projected change and least projected change. Grey and red colors indicate root depth truncation and blue indicates elongation.

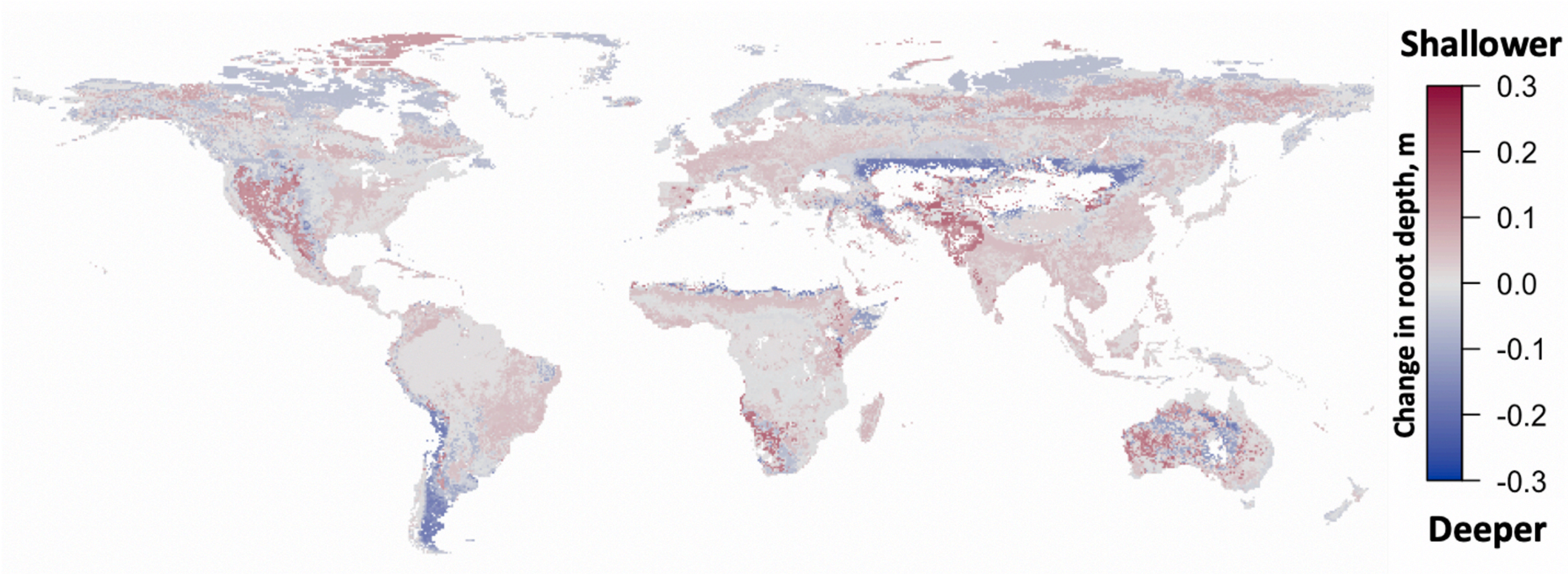

Figure S6. Change in depth to 50% rooting biomass (D50) due to differences in potential vegetation distributions compared to contemporary vegetation distributions. Red regions denote shallower roots in contemporary systems, while blue regions denote deeper roots in contemporary systems when compared to their potential vegetation distributions.

Table S1. Mean global rooting depth metrics with 95% confidence intervals for potential and contemporary land cover distributions under two scenarios of user assumptions. The third column displays the difference in cm between potential and contemporary root distributions and the percent change in parentheses. The first three rows indicate global means excluding true desert regions. The second three rows include true deserts in calculations of global mean rooting depth metrics but set roots in those systems to a depth of zero.

Metric	Potential Mean Rooting Depth (m, 95% CI)	Contemporary Mean Rooting Depth (m, 95% CI)	Change From potential (cm)
D99 (Desert excluded)	1.50 (+/- 0.001)	1.24 (+/- 0.00014)	-26 (17.3%)
D95(Desert excluded)	0.88 (+/- 0.00065)	0.73(+/- 0.00007)	-15 (17%)
D50 (Desert excluded)	0.07 (+/- 0.00007)	0.06 (+/- 0.000008)	-1 (14%)
D99 (Desert roots set to 0m)	1.32 (+/- 0.0011)	1.08 (+/- 0.00012)	-24 (18%)
D95(Desert roots set to 0m)	0.78 (+/- 0.0007)	0.64 (+/- 0.00007)	-14 (18%)
D50 (Desert roots set to 0m)	0.07 (+/- 0.00007)	0.05 (+/- 0.00008)	-2 (28.6%)