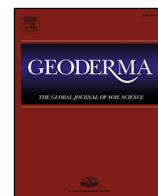




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Oxygen isotopes of phosphate and soil phosphorus cycling across a 6500 year chronosequence under lowland temperate rainforest

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ABSTRACT

Phosphorus (P) availability declines during ecosystem development due in part to chemical transformations of P in the soil. Here we report changes in soil P pools and the oxygen isotopic signature of inorganic phosphate ($\delta^{18}\text{O}_p$) in these pools over a 6500-year soil coastal dune chronosequence in a temperate humid environment. Total P declined from 384 to 129 mg P kg⁻¹ during the first few hundred years of pedogenesis, due mainly to the depletion of primary mineral P in the HCl-extractable pool. The $\delta^{18}\text{O}_p$ of HCl-extractable inorganic P initially reflected the signature of the parent material, but shifted over time towards (but not reaching) isotopic equilibrium. In contrast, $\delta^{18}\text{O}_p$ signatures of inorganic P extracted in water and NaHCO₃ (approximately 9 and 39 mg P kg⁻¹, respectively) were variable but consistent with isotopic equilibrium with soil water. In the NaOH-extractable P pool, which doubled from 63 to 128 mg P kg⁻¹ in the early stages of pedogenesis and then gradually declined, the $\delta^{18}\text{O}_p$ of the extracted inorganic P changed from equilibrium values early in the chronosequence to more depleted signatures in older soils, indicating greater rates of hydrolysis of labile organic P compounds such as DNA and increase involvement in P cycling as overall P availability declines through the sequence. In summary, this application of $\delta^{18}\text{O}_p$ to a long-term soil chronosequence provides novel insight into P dynamics, indicating the importance of efficient recycling through tight uptake and mineralization in maintaining a stable bioavailable P pool during long-term ecosystem development.

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1. Introduction

As an essential nutrient for life, from cellular structure to DNA and energetic pathways, the quantity and bioavailability of phosphorus (P) are important factors regulating ecosystem development. However, the percentage of the soil P that is readily bioavailable is typically a small fraction of the total P (Cross and Schlesinger, 1995; Hinsinger, 2001). The majority of soil P is present in organic forms, or bound with Al or Fe oxides in acidic soils, and with Ca in alkaline soils, all of which are considered to be poorly available to plants (Cross and Schlesinger, 1995; Trasar-Cepeda et al., 1990; Wagar et al., 1986).

A soil chronosequence is a series of soils that differ only in the time since the onset of their formation (i.e. parent material, vegetation, climate, and topography are held relatively constant). They are of significance because the space for time substitution allows observation of pedogenesis and associated ecosystem development over long time

spans (Stevens and Walker, 1970). Soil chronosequences are often characterized by a long-term decline in total soil P as the initial source of inorganic P in primary minerals is rapidly depleted (Crews et al., 1995; Olander and Vitousek, 2004; Wardle et al., 2004; Parfitt et al., 2005; Turner et al., 2007). This classic paradigm, defined by Walker and Syers (1976), has been generally accepted as the overall model for P transformations as soils age. The changes are twofold, as there is an overall decrease in P concentration due to leaching and erosion (Hedin et al., 2003) and a shift in the P forms that remain, with a decline in primary mineral P and an increase in organic P and inorganic P associated with secondary minerals (Crews et al., 1995; Parfitt et al., 2005; Turner et al., 2007). On stable landscapes, the long-term decline in soil P as ecosystems age can ultimately lead to a decline in plant biomass and productivity, termed ecosystem retrogression (Wardle et al., 2004).

The Haast chronosequence is composed of a series of coastal dune ridges located along the humid west coast of the South Island of New Zealand, north of the Haast River. Eger et al. (2011) and Turner et al. (2012a) provided a detailed description of the chronosequence together with soil chemical and physical data. In brief, the Haast chronosequence is a progradational dune system developed in coastal

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sands deposited following episodic earthquakes along the Alpine fault system. Soils are extremely acidic in all except the youngest soils and develop rapidly into Spodosols after about 2000 years (Turner et al., 2012a). Total P concentrations in mineral soil decline rapidly during the first few hundred years. Initially, inorganic P losses are partially offset by accumulations of organic and occluded P forms. However, total P continues to decline over longer periods of time, resulting in increasing C:P and N:P ratios in soil (Turner et al., 2012a).

One complicating factor when assessing P bioavailability is that P cycles quickly through various pools (Vitousek, 1984; Yanai, 1992). Moreover, because uptake and mineralization are tightly coupled, soil P concentrations alone are not sufficient to elucidate turnover rates. To shed more light on P transformations and bioavailability in soil along the Haast chronosequence, we analyzed the oxygen isotopes of inorganic phosphate ($\delta^{18}\text{O}_p$) in soil P pools extracted with increasingly strong chemical solutions (i.e. sequential fractionation). This approach has only recently been applied in soils using different extraction and purification methods (Zohar et al., 2010a, 2010b; Tamburini et al., 2010, 2012; Angert et al., 2011, 2012). In all of these studies, as in the current study, only the $\delta^{18}\text{O}_p$ of inorganic phosphate is determined. However, the tight coupling and cycling of P through organic and inorganic pools allow inferences to be made about the biogeochemical pathways involved, including those associated with organic P hydrolysis and utilization. Specifically, rapid intracellular P cycling via pyrophosphatase results in $\delta^{18}\text{O}_p$ values that reflect equilibrium with cell water and temperature (Paytan et al., 2002, Blake et al., 2005, Pfahler et al., 2013), while extracellular enzymes (e.g. acid or alkaline phosphomonoesterase, phosphodiesterase, or phytase) that catalyze the hydrolysis of organic P are expected to imprint kinetic isotopic signatures specific to each substrate and enzyme (Liang and Blake, 2006a, b; von Sperber et al., 2014).

Since there are multiple processes and environmental factors influencing the $\delta^{18}\text{O}_p$ signal, the interpretation of isotope data is most relevant when used in conjunction with other data, such as concentrations of organic and inorganic P pools, enzyme assays, and distribution of specific compounds as determined by ^{31}P NMR (McLaughlin et al., 2013; Paytan and McLaughlin, 2011). Given that the composition of organic P pools changes during the course of pedogenesis (McDowell et al., 2007; Turner et al., 2007, 2014) and since the bioavailability of organic P compounds can vary widely (Condon et al., 2005), $\delta^{18}\text{O}_p$ can provide information to help assess potential pathways of utilization and transformation for soil organic P.

2. Methods

2.1. Soil collection

Soils were collected from dunes Dune 1 (D1, 181 years before present), Dune 3 (D3, 392 years BP), Dune 8 (D8, 1826 years BP), Dune 11 (D11, 3384 years BP) and Dune 17 (D17, 6500 years BP) of the Haast chronosequence as previously described (Turner et al., 2012a). Four replicate samples were taken from a depth of 0–10 cm of the mineral soil from each of the sampled dunes between November 22 and 23 of 2010 and homogenized. Dune 2 (D2, 290 years BP) and Dune 12 (D12, 3903 years BP) were sampled in a similar way in 2012 and a sample from the organic layer (O horizon) of Dune 12 was also collected. Samples were dried at 30 °C, roots removed and soils ground and sieved to 2 mm.

2.2. Sample preparation and extractions

Phosphorus was sequentially extracted from the soil following a modification of the Hedley et al. (1982) method as described in Tiessen and Moir (1993). In brief, 3.3 g dried, ground and 2 mm-sieved soil was shaken overnight (~180–200 rpm, 16 h) with 200 mL of successive extraction solutions. Solutions used were Milli-Q water, 0.5 M

NaHCO_3 at pH 8.5, 0.1 M NaOH, and 1 M HCl. The following day, samples were centrifuged at 3500 rpm for 15 min and filtered (0.45 μm). Supernatant solutions were collected and aliquots taken for determination of inorganic P and total P concentrations. MgCl_2 was added to the remaining solution at a ratio of 2 g L^{-1} . The pH was raised to 10.5 to coprecipitate phosphate with magnesium hydroxide (MagIC) (Karl and Tien, 1992; McLaughlin et al., 2004). The concentration data is provided in Supplementary Table 1.

The Hedley procedure is a commonly used method for separating soil P into fractions based on chemical extractability, with those fractions then being assigned a degree of bioavailability (Cross and Schlesinger, 1995). There are many criticisms of the method, but its widespread use allows comparisons to be made among contrasting soils, climates, and land-uses if its limitations are acknowledged (Condon and Newman, 2011). In particular, soil P pools are operationally defined and chemical extractability can be poorly related to bioavailability. Indeed, the various pools overlap along a continuum of potential bioavailability, from water-soluble P (most available) to a mild acid solution (moderately available) and non-extracted (residual) P, which is considered to be of limited availability (Condon and Newman, 2011). We note that microbial P was not analyzed separately because the soil samples were shipped dry. However, we expect that this fraction is included predominantly in the water, NaHCO_3 , and NaOH pools of the Hedley extraction (Turner et al., 2003, Turner and Haygarth, 2003).

2.3. Phosphorus determination

Aliquots of sequential extracts were analyzed for soluble reactive phosphate (SRP – the molybdate reactive P, which includes primarily free orthophosphate plus any polyphosphates and possibly some organic P degraded in the acid reaction; referred to in this manuscript as inorganic P) (Grasshoff et al., 1983) after pH adjustment as described in Tiessen and Moir (1993). Samples were either processed individually using a spectrophotometer [Schimadzu UV-1201] or in a proportionally scaled down version on a microplate analyzed on a plate reader [Molecular Devices SpectraMax M2e] (Briggs et al., 2013). The lower limit of detection was 3.1 $\mu\text{g P L}^{-1}$, and standard deviations were up to $\pm 3.1 \mu\text{g P L}^{-1}$. Total P concentrations were analyzed on an ICP-OES [Perkin-Elmer Optima 4300 DV Inductively Coupled Plasma Optical Emission Spectrometer operated by the University of California, Santa Cruz]. Water extracts were analyzed without dilution, while the 0.5 M NaHCO_3 , 0.1 M NaOH, and 1 M HCl extractions were diluted five-fold to minimize the impact of matrices on the plasma in the ICP-OES. The detection limit on this instrument for the two P channels is 11 $\mu\text{g P L}^{-1}$ with sample replicates within $\pm 0.1 \text{ mg P L}^{-1}$, the calibration ranged between 1.25 and 5 mg P L^{-1} corresponding to the range of concentrations in the samples. Measured blanks were always less than 1% of signal. Total P in the sample was calculated by adding the total P concentration of all fractions in the Hedley extraction and thus may underestimate the actual soil total P if some refractory P is present in the insoluble residue. The soluble unreactive P (SUP – the molybdate unreactive P, which is referred to in this paper as organic P – although it also includes the inorganic non-acid labile polyphosphates) was calculated from the difference between total P and SRP.

2.4. Analysis of $\delta^{18}\text{O}_p$

Silver phosphate (Ag_3PO_4) was prepared for isotope analysis following a modification of the procedure described by Zohar et al. (2010a). In brief, the MagIC precipitate was dissolved in 5 mL of 17.4 M acetic acid and the smallest amount of 10 M HNO_3 needed to dissolve the brucite precipitate. To remove dissolved organic matter, one of two methods was used: (1) solutions were brought up to pH 5.0 and shaken with DAX 8 resin, or (2) solutions were brought to a pH of 1, 0.2 μm filtered, and passed through an Oasis HLB column. Sample pH was brought up to

5.5 and cerium nitrate added to precipitate cerium phosphate. The remainder of the process follows the method described in McLaughlin et al. (2004) and Zohar et al. (2010a). Sample splits were processed with ^{18}O enriched reagents as described in McLaughlin et al. (2006) to determine if oxygen exchange occurs during processing and no measurable differences between the spiked and un-spiked samples were detected. Silver phosphate samples were processed at the US Geological Survey Stable Isotope Laboratory in Menlo Park, CA, using a Eurovector Elemental Analyzer connected to Micromass Isoprime mass spectrometer. Internal standards (calibrated using fluorination) with values of $11.3 \pm 0.15\%$ and $20.0 \pm 0.25\%$ were used for mass and drift corrections, sample standard deviation averaged $\pm 0.3\%$. Details about the equipment and standards are described in McLaughlin et al. (2004).

2.5. Analysis of soil water $\delta^{18}\text{O}$

Soil samples from two dunes (Dune 8, 1826 years BP and Dune 2, 290 years BP) were collected in 2012 for analysis of $\delta^{18}\text{O}$ of soil water. Soil water was extracted from soil plugs that were kept frozen until analysis using a modified method from West et al. (2006). Samples were cryogenically distilled in a vacuum line and then analyzed as discrete samples using a Picarro L230-I water isotope analyzer (at the University of California Santa Cruz). Water standards ranging between -29% and 0% were used and the standard deviation was 0.18% and reproducibility among replicates was 0.5% .

3. Results

3.1. Distribution of P in Hedley fractions

For the water and NaHCO_3 extractions, no significant trends in the absolute concentrations of P were observed over the entire chronosequence (around 9 and 39 mg P kg^{-1} , respectively; Fig. 1a), although their relative proportional contribution increased slightly after the first few hundred years of pedogenesis (Fig. 1b). The NaOH total P doubled during the first few hundred years of pedogenesis (from 63 to 128 mg P kg^{-1}) and then decreased slightly over the remainder of the chronosequence. This is reflected by NaOH total P

constituting an increasing proportion of the total P pool along the chronosequence, becoming the dominant pool of P ($\sim 60\%$) after the first few hundred years of pedogenesis (Fig. 1b). The HCl-extractable total P concentrations were greatest initially ($\sim 60\%$ of the total P) and declined dramatically over the first few hundred years of the chronosequence, from ~ 260 to 10 mg P kg^{-1} (Fig. 1). The sum of total P of all pools for each dune was calculated and considered as the total P for the soil sample, ranging from 384 mg P kg^{-1} initially to 133 mg P kg^{-1} after 6500 years (Supplementary Fig. S1).

Inorganic P concentrations (shown both as absolute and relative values in Fig. 2) constituted about 80% of the total P in the first few hundred years (primarily in the HCl extracted fraction) but decreased to approximately 50% over the next ~ 2000 years and then declined slightly further to 40% over the remainder of the chronosequence (Fig. 2b). The water and NaHCO_3 -extracted pools remained relatively constant over time, although their relative proportion increased slightly by the end of the chronosequence due to the decreasing contribution of the other pools. The NaOH pool increased during the first few thousand years then decreased slightly over the remainder of the chronosequence. In contrast, the HCl pool declined rapidly in the first few hundred years, and then remained constantly low throughout the remainder of the chronosequence.

Organic P concentrations were small in all the fractions except the NaOH pool (Fig. 3a). In water extracts, organic P remained at a relatively constant concentration and relative proportion throughout the 6500-year chronosequence. The NaHCO_3 pool fluctuated between 10 and 40 mg P kg^{-1} , but showed no clear trend over time. Organic P in NaOH extracts increased along the chronosequence, both in concentration and relative proportion. The organic P pool in the HCl extraction was negligible throughout the sequence.

3.2. $\delta^{18}\text{O}_\text{P}$

The $\delta^{18}\text{O}_\text{P}$ of inorganic phosphate in water and NaHCO_3 extracts fluctuated between 15.2% and 20.9% along the chronosequence (Fig. 4), but remained close to the expected equilibrium values with soil water and temperature (16% to 24% , see below). The HCl-extractable phosphate had the lowest $\delta^{18}\text{O}_\text{P}$ values, ranging from 8.6% to 13.9% . The

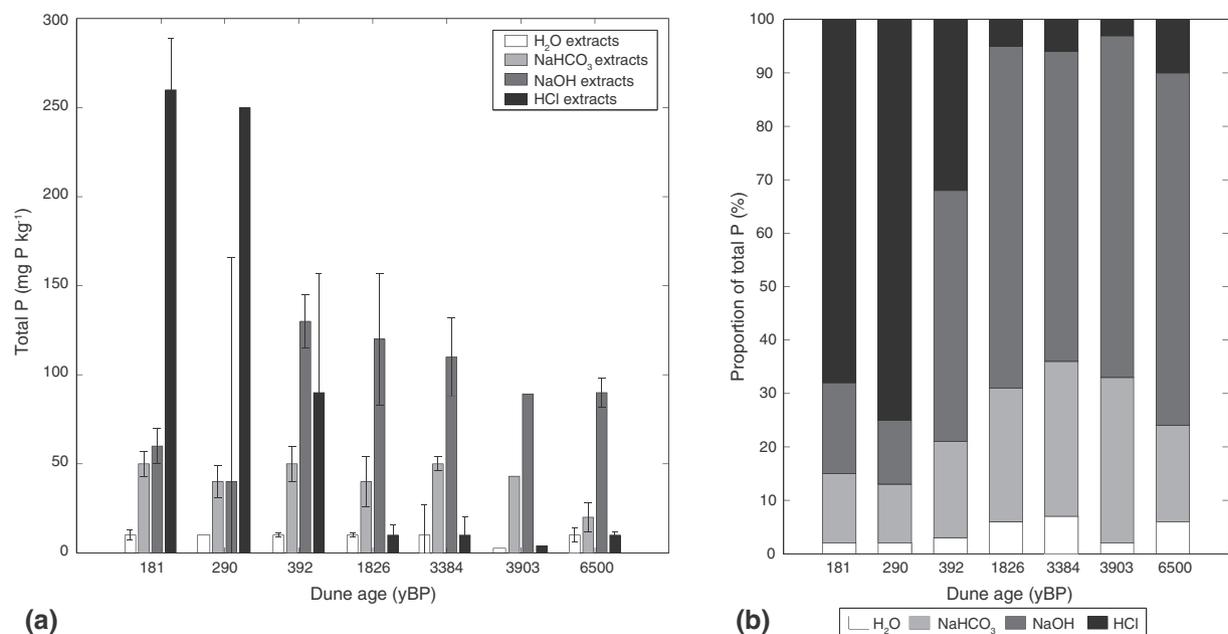


Fig. 1. Total P concentrations in units of mg of P per kg of soil (a) and relative proportions (b) within each extraction solution across the chronosequence. The standard deviation in panel a is for replicate ($n=2$ or 3) soil samples extracted.

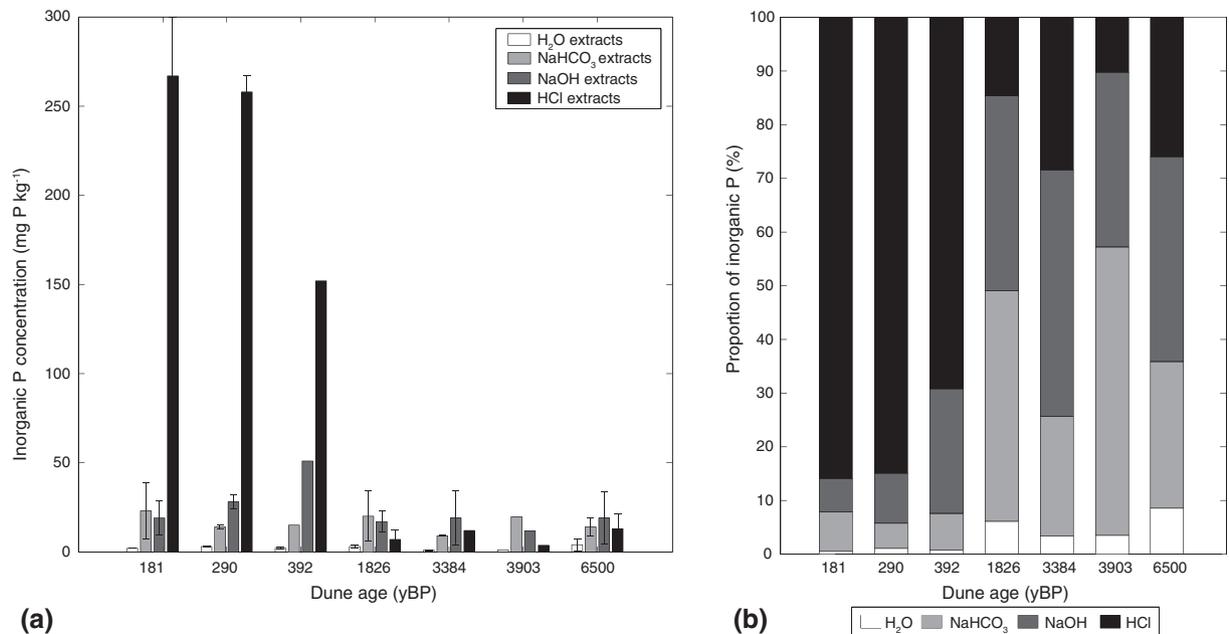


Fig. 2. Inorganic P concentrations in units of mg of P per kg of soil (a) and relative proportions (b) within each extraction pool across the chronosequence. The standard deviation in panel a is for replicate soil samples extracted.

$\delta^{18}\text{O}_p$ of the NaOH-extractable phosphate was initially similar to that of the water and NaHCO₃ values, but then decreased by approximately 5‰ in the remainder of the chronosequence.

3.3. Equilibrium calculations

To determine how close the $\delta^{18}\text{O}_p$ of the various fractions was to isotopic equilibrium values with soil water at environmental temperature, we calculated the range of possible $\delta^{18}\text{O}_p$ equilibrium values using soil temperatures and soil water isotope ratios ($\delta^{18}\text{O}_w$) (Longinelli and Nuti, 1973). We used the annual average soil temperature (12.9 °C) as well as average low (9.6 °C) and high (16.2 °C) temperatures (Barringer and Lilburne, 2000) and average (0.2‰), lowest

(−2.75‰) and highest (0.38‰) values we measured for the soil water isotopes, to calculate the equilibrium values using the equation: $\delta^{18}\text{O}_p = [(111.4 - T) / 4.3] + \delta^{18}\text{O}_w$, where T is the environmental temperature in °C, and $\delta^{18}\text{O}_p$ and $\delta^{18}\text{O}_w$ are the isotopic composition of phosphate and water, respectively (rearranged from Longinelli and Nuti, 1973) (dark gray shaded region in Fig. 4). The range determined is between 24‰ and 19‰ (average 21.7‰). However it is likely that the actual range is larger because the range of soil water isotope ratios in this environment might not be captured by our limited soil water data (soil from two dunes collected over only two days). Specifically, since the soil samples for $\delta^{18}\text{O}_w$ analysis were collected during the warm summer season, we expect that they are enriched compared to the annual average. Moreover, some evaporation likely occurred during

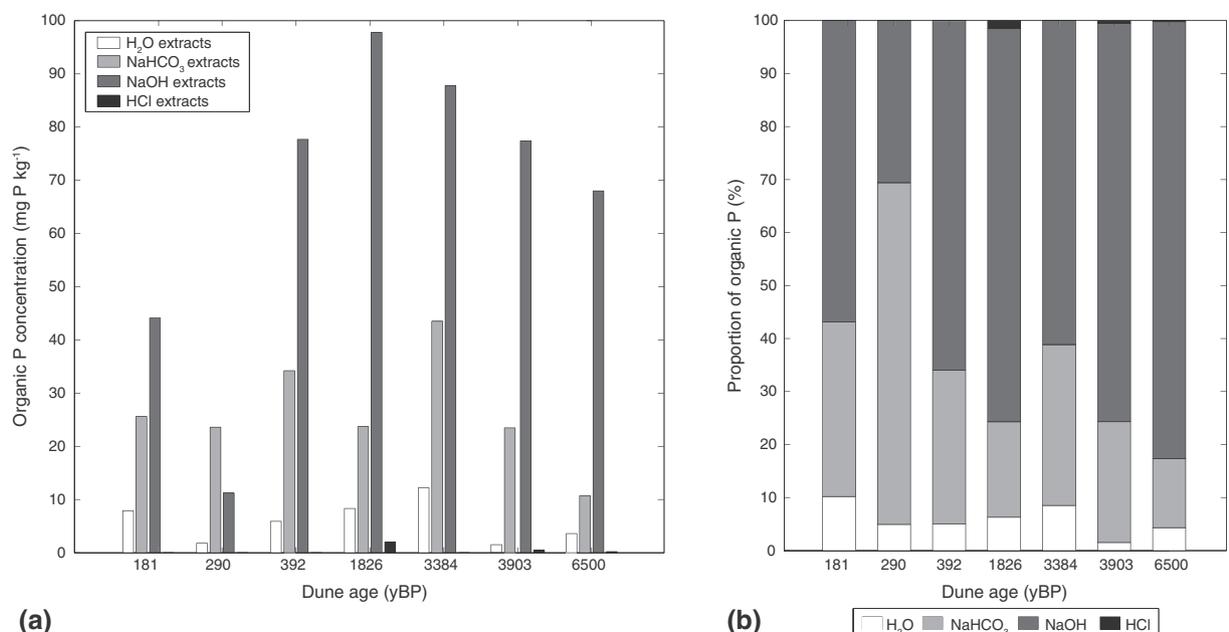


Fig. 3. Organic P concentrations in units of mg of P per kg of soil (a) and relative proportions (b) in the Hedley extraction solutions for each dune analyzed.

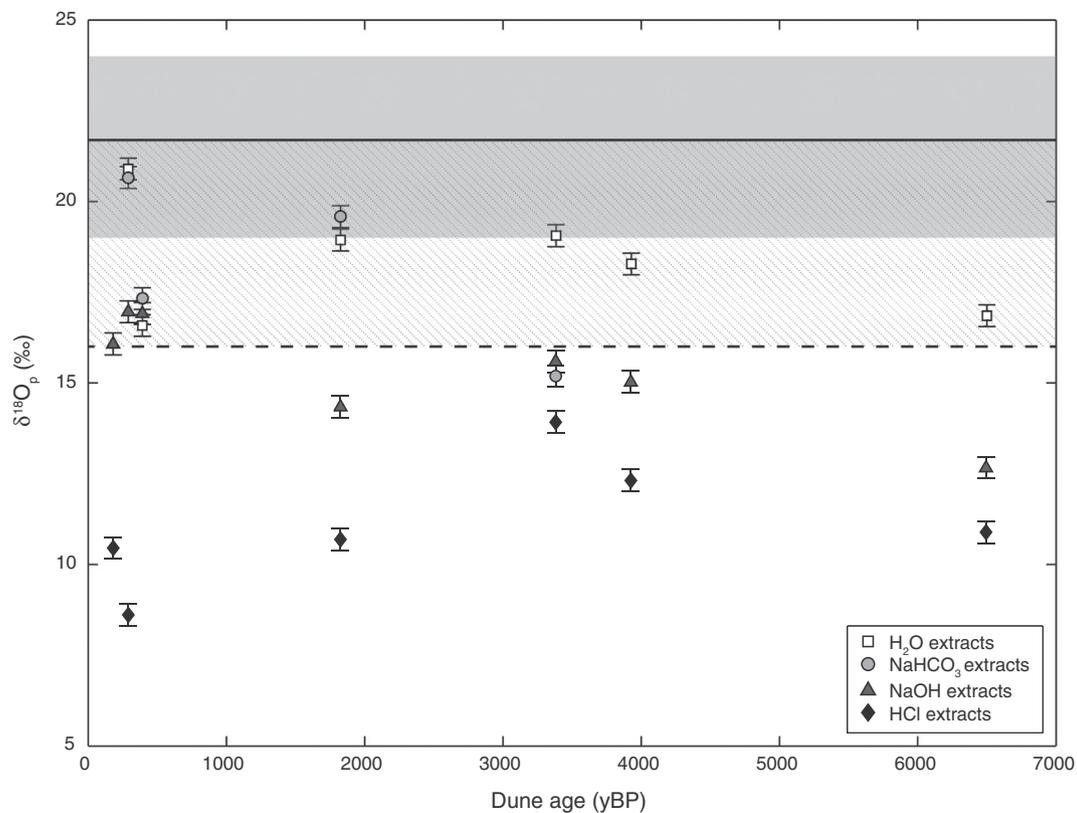


Fig. 4. $\delta^{18}\text{O}_p$ signatures in different soil P fractions over time. The error bars of 0.3‰ are based on the analytical reproducibility of the silver phosphate method described in McLaughlin et al. (2004). The dark shaded area (between 24‰ and 19‰) represents equilibrium range calculated based on the average low (9.6 °C) and high (16.2 °C) soil temperatures (Barringer and Lilburne, 2000) and lowest and highest soil water isotopes values ($\delta^{18}\text{O}_w$) measured (−2.75‰ and 0.38‰, respectively). The solid line is the equilibrium value calculated the annual average soil temperature (12.9 °C) and average $\delta^{18}\text{O}_w$ (0.2‰). The dashed line is the equilibrium value calculated using average soil temperature and a $\delta^{18}\text{O}_w$ of −5.5‰, which is ~2‰ enriched with the heavier oxygen isotopes compared to local rainwater values and the dotted area represents equilibrium values that encompass this value (see text for details). Equilibrium values were calculated using the equation $\delta^{18}\text{O}_p = [(111.4 - T) / 4.3] + \delta^{18}\text{O}_w$ where T is the environmental temperature in °C, and $\delta^{18}\text{O}_p$ and $\delta^{18}\text{O}_w$ are the isotopic composition of phosphate and water respectively (rearranged from Longinelli and Nuti, 1973).

shipping and processing. Accordingly, we also calculated an expected equilibrium value using the average soil temperature and a $\delta^{18}\text{O}_w$ of −5.5‰, which is ~2‰ enriched with the heavier oxygen isotopes compared to local rainwater values (IAEA station Invercargill). This 2‰ enrichment of soil water in the upper 10 cm of soil relative to precipitation is typical under similar soil characteristics (humidity, grain size) and climate conditions (Gazis and Feng, 2004; Hsieh et al., 1998).

4. Discussion

As expected, total P along the Haast chronosequence declined by approximately 70% from 384 mg P kg^{−1} to 129 mg P kg^{−1} over the ~6500 year sequence (Crews et al., 1995; Hedin et al., 2003; Parfitt et al., 2005; Turner et al., 2007). These values are similar to values for total P measured previously by acid digestion (Turner et al., 2012a, 2012b) (supplementary material). The small differences are likely due to the different methods used to calculate total P and the different depths homogenized and analyzed for each sample (this study used the 0–10 cm depth of the mineral soil, while Turner et al. (2012a, 2012b) analyzed a homogenized sample to 20 cm).

Along the chronosequence, total P in the water and NaHCO₃-extractable pools showed no clear trend with time. This is likely maintained by tight coupling between uptake and replenishment of P in these highly accessible pools, as well as by interactions with more stable soil P pools. It is however difficult to determine where the P that replenishes these pools originates from. Based on changes in P concentrations of the other pools (e.g. NaOH and HCl fractions), the water and NaHCO₃ pools may be initially supplied from the HCl soluble fraction as this pool decreases during the first few hundred years of the chronosequence

(Figs. 1 and 2). The decrease in the inorganic P concentrations of the NaOH pool between 392 and 1826 years BP could indicate that this fraction is also contributing P to the more labile pools, although the labile pools are more likely maintained through continuous mineralization of organic P. An additional possibility is that the constant inorganic P concentrations in the water and NaHCO₃ pools are maintained by rapid uptake and recycling of these labile inorganic pools with minimal loss from the system, particularly as soils age.

The isotopic signatures of these bioavailable pools were close to the expected equilibrium range with soil water at environmental soil temperatures. This indicates rapid P cycling, whereby organisms have processed P intracellularly using the pyrophosphatase enzyme at a rate that is much faster than new P is added to these pools. This intracellular P metabolism effectively erases any isotopic signature of the source phosphate and is consistent with the low concentrations of P in the water and NaHCO₃-extractable pools and indicative of rapid uptake and mineralization. This is also consistent with laboratory experiments that show fast equilibration of labile P pools in soils, on the order of weeks (Zohar et al., 2010b) and with similar studies along a chronosequence in Switzerland (Tamburini et al., 2012) and in soils from Israel (Angert et al., 2011). The variability observed between dunes is also consistent with rapid cycling, as it is likely that the soil temperature and water isotopes vary among dunes and with time.

Interestingly, while within the expected range of values that represent equilibrium with soil water, most $\delta^{18}\text{O}_p$ measurements in the water and NaHCO₃ pools lie towards the lower range of the equilibrium values. Assuming that the soil water isotope ratio of −5.5‰ is representative of the annual average value, this indicates that some labile P originates from a source with an isotopic signature that is

lower than that expected from equilibrium with soil water. Early in the sequence, this source could be HCl-extractable P with an isotopic signature of ~10‰. Based on simple isotope mass balance calculations, the unchanged mineral contribution (e.g. with an isotopic signature of 10‰) would constitute less than 10% of the total P in the water-soluble or NaHCO₃ extracted fractions (reducing the signature by ~1‰). However, this only explains data from the first few hundred years of the sequence when the HCl-extractable P is decreasing in concentration and has low isotope ratio of ~10‰. The isotopic signature of the labile fractions for the older dunes is also at the low range of values consistent with equilibrium with soil water. For these older dunes it is more likely that this trend is indicative of P that has originated from mineralization of organic P compounds via extracellular enzymes. This is consistent with organic P becoming the largest P pool in the soil and hydrolysis of organic P typically imprints an isotopic signature on the phosphate produced by hydrolysis, which is lower than that expected for equilibrium (Liang and Blake, 2006a,b; von Sperber et al., 2014). The consistent equilibrium isotope ratios indicate that despite the relative stability in concentration of the most bioavailable fractions (water and NaHCO₃ pools), these pools are not stagnant but rather are shaped by rapid mineralization and uptake by soil microbes, even in older soils (Yanai, 1992).

The dramatic decline in HCl-extractable P, from 262 mg P kg⁻¹ to less than 82 mg P kg⁻¹ over the first few hundred years of the sequence (Fig. 2), indicates that most of the P loss at the early stages of the chronosequence is from primary mineral P (Walker and Syers, 1976). Some of this P is completely lost from the system due to leaching and mobilization by water and erosion of soil particles, and some is sequestered into plant biomass and moved into the organic layer (Turner et al., 2012a). The δ¹⁸O_p of the HCl-extracted fraction for the younger dunes is close to 10‰, which likely represents the bedrock signature and is consistent with expected values for metamorphic and igneous rocks ranging from 7.5 to 15‰ (Taylor and Epstein, 1962). The HCl-extracted fraction shifts towards higher values (increasing by ~3‰ and getting closer to equilibrium) as the soils age, suggesting some precipitation or occlusion of phosphate from soil water into this fraction over time, although not at a rate fast enough to attain full equilibration with soil water within the frame represented by the chronosequence.

A similar result was obtained from the 150 year Damma chronosequence in the Swiss Alps (Tamburini et al., 2012), where the δ¹⁸O_p of HCl-extractable P initially reflected the bedrock value, but older soils in the same area matched the calculated equilibrium values along the chronosequence (Tamburini et al., 2012). The HCl-extractable P of old soils in Israel was also consistent with expected equilibrium (Angert et al., 2011). All these studies suggest that the moderately labile HCl fraction is gradually transformed through dissolution (aided by the soil acidity) and re-precipitation of new secondary minerals that form from phosphate having undergone biological cycling, eventually approaching isotopic equilibrium (Tamburini et al., 2012). Indeed, laboratory experiments with fertilized agriculture soils, where soil microbial activity is high, suggest that the isotopic signature of the HCl-extracted P fraction can be modified even on time scales much shorter than those captured in the studied chronosequences (Zohar et al., 2010a, 2010b). Interestingly, the isotope value of the HCl-extracted P fraction of oldest soil from the Haast chronosequence decreases again, perhaps explained by addition of detrital apatite from mineral dust addition to the soil as suggested by Eger et al. (2013). The source of the dust is local metamorphic rock mostly of igneous origin (Eger et al., 2013) and it is likely to maintain the protolith's δ¹⁸O_p signature in apatite (e.g. values of 7–12‰).

The NaOH-extractable inorganic P is considered to be composed of moderately labile non-occluded P adsorbed on Al and Fe oxides and hydroxides (Parfitt, 1989). The concentration of NaOH-extractable inorganic P increased over the first few hundred years of the chronosequence and then remained constant (Figs. 1–3). The δ¹⁸O_p of this pool was initially close to isotopic equilibrium with soil

water (similar to the more labile water and NaHCO₃ fractions), but gradually decreased along the chronosequence to values ~4‰ lower than equilibrium. This indicates that the NaOH-extractable inorganic P is actively involved in chemical, physical, and/or biological transformations throughout the chronosequence (Cross and Schlesinger, 1995; Schoenau et al., 1989; Trasar-Cepeda et al., 1990; Wagar et al., 1986). However, the observation that the NaOH-extractable inorganic P did not attain isotopic equilibrium suggests that its turnover rate by soil organisms or plants is relatively slow (or slower than the uptake, utilization and release by the biomass and slower than the water and NaHCO₃ pools).

The direction of change in the δ¹⁸O_p of the NaOH-extractable phosphate can shed light on the processes that shape this pool. There are two potential sources of phosphate with a lower than equilibrium isotopic signature that could be incorporated into the NaOH inorganic P pool (e.g. adsorbed by Al and Fe oxides and hydroxides in the soil) at Haast. The first source of low δ¹⁸O_p is P released from the mineral fraction (e.g. the HCl extracted fraction), which has an isotope ratio around 10‰ early in the chronosequence. Indeed, the rapid decrease in inorganic P of the HCl pool (Fig. 2) and the parallel increase in P of the NaOH pool are consistent with this interpretation. A decrease in δ¹⁸O_p of the NaOH inorganic P fraction by about 3‰ suggests the addition of 17 mg P kg⁻¹ of soil from the HCl pool (using simple mass balance). This is within the 10 to 20 mg P kg⁻¹ increase in NaOH-extractable inorganic P from 181 to 392 years (from about 20 to about 50 mg P kg⁻¹; Fig. 2). However, the P concentration in the HCl-extracted fraction is stable after the first few hundred years of pedogenesis. Hence, although this may be the source of P that adsorbs to Al and Fe oxides early in the chronosequence, this process cannot explain the change in δ¹⁸O_p observed later along the chronosequence, when the δ¹⁸O_p of the NaOH decreases but no changes in the HCl fraction is observed.

Phosphate with a low δ¹⁸O_p signature can be produced by hydrolysis of organic P mediated by extracellular enzymes (Liang and Blake, 2006a, b; von Sperber et al., 2014). This process imprints non-equilibrium signatures on the product phosphate, typically shifting δ¹⁸O_p towards lower than equilibrium values (Liang and Blake, 2006a,b; McLaughlin et al., 2013; Paytan and McLaughlin, 2011; von Sperber et al., 2014). It is thus possible that some of the phosphate released from organic matter hydrolysis in the soil is rapidly adsorbed onto Al and Fe oxides before it is recycled to attain equilibrium values. Recent ³¹P NMR work found that most forms of organic P at Haast initially increase in concentration during the early stages of pedogenesis, and then decline over time (Turner et al., 2014). Specifically, phosphomonoesters constitute 80% of the organic P in the Haast soils, of which the most abundant are inositol hexakisphosphate stereoisomers (Turner et al., 2014). It is possible that the hydrolysis of phosphomonoesters contribute to the NaOH extracted inorganic P fraction, resulting in the lower than equilibrium signature we see in the older dunes. Phosphate that originates from hydrolysis of monoesters via acid phosphatase enzymes incorporates oxygen with an isotopic fractionation of about -10‰ (von Sperber et al., 2014). During such hydrolysis, one oxygen atom is incorporated from water, thus this oxygen is expected to have an isotope value of -15‰ (for soil water of -5‰). The other three oxygen atoms are retained from the organic P (e.g. inherited).

Assuming the organic matter is from the organic horizon the signature of these oxygen atoms is around +28‰, based on our analysis of the water extractable P fraction of the organic matter rich O horizon, the product P will have an isotopic signature of around 17‰, similar to the signature expected from equilibrium. This specific process cannot therefore explain the observed trend of the decrease in the δ¹⁸O_p throughout the chronosequence. It is possible that despite the fact that phosphomonoesters comprise the majority of the soil organic P (e.g. 51–66% in young soils and 69–75% in the older soils), other more labile organic P pools such as phosphodiester, specifically DNA, with lower abundance (e.g. 4–8%) (Turner et al., 2014) are hydrolyzed more readily and that phosphodiester hydrolysis is responsible for the

observed below equilibrium values (and the low phosphodiester concentrations). For example, during the kinetic fractionation involved in the hydrolysis of DNA by deoxyribonuclease (DNase) and acid phosphatase, two oxygen atoms are retained from DNA (presumably with an equilibrium signature of 28‰), the third oxygen hydrolyzed by DNase involves a fractionation of -20% and the fourth hydrolyzed by acid phosphatase a fractionation of -10% relative to water. A soil water isotopic signature of -5% will therefore produce inorganic phosphate with an isotopic signature of 4‰. Based on simple mass balance calculations, for a NaOH inorganic P pool that started close to equilibrium (17‰), in order to get a signature of approximately 13‰, about 30% of the inorganic P in this pool would have originated from the hydrolysis of DNA. Indeed, phosphodiester are weakly sorbed in soils and turn over more rapidly than monoesters such as inositol phosphates (Condrón et al., 2005; Celi and Barberis, 2007).

Accumulation of DNA in older soils (as a proportion of the total organic phosphorus) has been speculated to result from either incorporation into recalcitrant soil organic matter (Turner et al., 2007), or from increasing importance of soil microbial biomass as soils age (Turner et al., 2013; Vincent et al., 2013). Our data is consistent with an actively cycling DNA pool, suggesting it is not sequestered into recalcitrant organic matter and therefore pointing to an increase in importance of the microbial biomass with age. Hydrolysis of DNA might also explain the trend towards the lower range of equilibrium for some of the water and NaHCO_3 inorganic P fractions (e.g. instead of 10% contribution from the bedrock, an approximate 5% input of phosphate from DNA hydrolysis may explain the data). Further investigation into the isotopic signatures of various organic P pools in conjunction with enzyme assays or incubation experiments would be interesting and effective ways to further elucidate the biogeochemical pathways and cycling of organic P compounds occurring across this chronosequence, and would improve the interpretation of the isotope trends we observe, particularly for the NaOH inorganic P fraction.

Our investigation using Hedley fractionation and $\delta^{18}\text{O}_\text{p}$ of the various extracted fractions along the Haast chronosequence revealed processes that are difficult to infer from just measurement of concentrations of total organic and inorganic P concentrations. The findings of this study indicate that turnover of organic P, and specifically DNA, is likely to contribute P to the labile (water and NaHCO_3) and semi-labile (NaOH) pools. In addition, we note that despite the dramatic decrease in total P and inorganic P during pedogenesis, the more labile bioavailable pools (water, NaHCO_3 , the inorganic fraction of the NaOH extracted pool) remain relatively constant in concentration and their isotopic signature reflects continuous and extensive cycling via the soil microbial biomass.

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