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The effects of temperature on soil phosphorus availability and phosphatase enzyme activities: a cross-ecosystem study from the tropics to the Arctic

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Abstract Earth system models predict large increases in global terrestrial net primary productivity (NPP) over the next century, largely reflecting positive effects of climate change and increasing atmospheric carbon dioxide concentrations on plant growth. However, while theory predicts that soil phosphorus (P) availability may keep pace with P demand as the climate warms, we lack experimental evidence to support this prediction. Here, using a set of laboratory experiments and incubations, we measured both the effect of temperature on the mechanism of biochemical P mineralization—phosphatase (Ptase) enzyme activities—and on rates of soil P mineralization in soils from a range of ecosystem types from the tropics to the Arctic. Consistent with temperature effects on soil nitrogen (N) mineralization, we found that both Ptase activities and P availability in soil increased with temperature following macromolecular rate theory (MMRT) based kinetics. However, across all sites and temperatures, there was no relationship between Ptase activity and mineralized P, indicating that the potential responses of P mineralization with warming vary among ecosystems. The lack of relationship between Ptase and P availability with increasing temperature is consistent with previous work showing that P mineralization rates are also strongly affected by other biotic and abiotic factors, including organic P substrate availability and the geochemical properties of soil. However, our results indicate that the use of Ptase temperature kinetics alone as a proxy for soil P mineralization in terrestrial ecosystems is insufficient to predict future P availability accurately, and modeling efforts that do so will likely overestimate the effects of temperature on soil P availability.

Keywords P mineralization · Phosphatase enzymes · Soil enzymes · Climate change · Enzyme kinetics

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Introduction

Many Earth system models (ESMs) predict that increases in atmospheric carbon dioxide (CO_2) concentrations will cause a strong plant fertilization effect, driving potentially large increases in net primary productivity (NPP) and carbon (C) storage in terrestrial ecosystems over the next century

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(Ahlström et al. 2015). This prediction is borne out in many terrestrial ecosystems, at least in the short term, where experimental increases in CO_2 concentrations stimulate NPP across a range of ecosystem types (Norby et al. 2005). At the global scale, however, the extent to which CO_2 will enhance NPP—and for how long—remain subjects of considerable debate.

At the heart of this debate are uncertainties about other potential constraints on terrestrial NPP, particularly the extent to which low nutrient availability may constrain future productivity. NPP in many terrestrial ecosystems is already thought to be nutrient limited (LeBauer and Treseder 2008; Vitousek et al. 2010; Hou et al. 2020). Indeed, stand level responses to CO_2 fertilization are tightly linked to nitrogen (N) availability (Reich et al. 2014), and there is evidence of similar constraints by phosphorus (P) (Ellsworth et al. 2017). Following this logic, Wieder et al. (2015) used a stoichiometric approach to assess possible nutrient constraints on terrestrial NPP and C storage projections in the suite of ESMs used in the 5th iteration of the Coupled Model Intercomparison Project (CMIP-5). Their analyses not only suggested the potential for substantial decreases in CO_2 fertilization effects on NPP, but that those decreases could be sufficient to convert terrestrial ecosystems from a net C sink to a net C source by 2100.

While the theoretical basis for potential nutrient constraints on future NPP is clear, some argued that the findings of Wieder et al. (2015) underestimated the future availability of N and P by insufficiently addressing the possible effects of global climate warming on nutrient input and internal mineralization pathways (Brovkin and Goll 2015). With projected temperature increases in global MAT of 3–5 °C over the next century (Wieder et al. 2015), such effects may have profound impacts on nutrient cycling and availability in many terrestrial ecosystems. However, until now, no study has directly assessed the effects of temperature on soil P mineralization rates across ecosystems or evaluated the relationship between the temperature responsiveness of P mineralization together with its primary, underlying mechanism, soil Ptase activities.

Multiple lines of direct evidence suggest that ecosystem N availability may keep pace with elevated demand from increasing NPP. Additional N inputs may come from N fixation, N deposition, weathering of rock N, or through accelerated rates of soil N mineralization with warming) (Ceuterick et al. 1978; Gurevitch 2001; Houlton and Morford 2015; Liu et al. 2016). Indeed, by way of meta-analysis, Rustad et al. (2001) showed an average 46% increase in in rates of net N mineralization with warming (0.3–6.0 °C) across a range of ecosystem types. By contrast, mineral P weathering is the dominant and only significt source of new P to ecosystems. While rates of mineral P weathering may increase slightly with warming (Goll et al. 2014), the relatively slow increases are expected to be most pronounced in high latitude ecosystems where there is little evidence of P limitation (Vitousek et al. 2010; Goll et al. 2014). Similarly, while increases in P deposition may increase with global land use change and desertification, new inputs of P via are predicted to be very modest and spatially heterogeneous (Wang et al. 2017).

As with N, even absent significant inputs of new P inputs, climate warming-driven increases in plant and microbial P mineralization of soil organic P (P_o; P mineralization) could still significantly enhance soil P supply (e.g., Brovkin and Goll 2015). However, unlike N, direct empirical evidence of enhanced soil P mineralization with warming is sparce, limited to a handful of investigations in individual ecosystems that used now-outmoded methods (Bünemann 2015) and that produced equivocal results (Nadelhoffer et al. 1991; Jonasson et al. 1993; Grierson et al. 1999). While some have argued that increases in P mineralization may parallel observed increases in soil N mineralization with warming (Brovkin and Goll 2015), critical mechanistic differences between the two processes nessecitate an independent investigation of the effects of temperature on soil P mineralization.

While most N mineralization occurs during decomposition of C-bonded N (biological mineralization), soil P tends to be stabilized in organic matter as phosphate esters. As a result, P mineralization in soils is primarily a biochemical process (McGill and Cole 1981), catalyzed by extracellular enzyme produced by plants and microbes, the investment in which is upregulated under conditions of P limitation (Olander and Vitousek 2000; Treseder and Vitousek 2001). Thus, rates of P mineralization are hypothesized to increase with temperature as the activities of phosphatase enzymes become more efficient (Wallenstein

et al. 2011), and this prediction is reflected in the representation of P mineralization in ESMs (Wang et al. 2007; Wang and Houlton 2009). However, while this prediction makes sense conceptually, the link between the kinetics of potential Ptase enzyme activities and soil P mineralzation remains unknown. Here, we tested the hypothesis that soil warming will enhance soil P availability by accelerating rates of P_o hydrolysis via extracellular phosphatase enzymes, resulting in increased rates of P mineralization in soils. To do this, we used a set of soil incubations in the laboratory, evaluating the response of soil P mineralization rates and potential phosphatase enzyme activities to temperature in soils from a range of ecosystem types.

Materials and methods

Study sites, soil sampling and analysis

To assess temperature controls on soil P dynamics, we sourced soil samples from eight different sites (Fig. S1), spanning latitude (from 8.4° to 63.7° N), soil type, vegetation type, and climate conditions (Table 1). At each site, five mineral soil cores were taken 1 m apart along each of three radial transects distributed evenly about a shared point of origin. Soils were sampled to 10 cm depth using an 8 cm diameter bulb corer (or equivalent), homogenized by hand, and stored at ~ 4 °C before analysis and/or transport. Soils were transported to Missoula, MT, USA within 48 h of sampling, and subsampled for analyses upon arrival. Soils for nutrient analyses and incubations were stored at for 4 °C for at least 12 h before analysis or incubation. Soils used to measure Ptase activities were stored at − 20 °C until analysis.

Soil gravimetric water content was measured by water mass loss after drying for 48 h at 105 °C and soil water holding capacity was determined by measuring gravimetric soil moisture on well-drained soils after wetting. To measure soil pH, we shook 10 g of oven-dried equivalent field fresh soil in 20 ml 0.02 M $CaCl_2$ using an orbital shaker at 150 RPM for 30 min, equilibrating for 10 min, and measuring pH with an Accumet AP72 pH/mV/temperature handheld meter (ThermoFisher Scientific, Waltham, MA). We measured initial soil resin P concentrations by shaking 10 g field fresh soil subsamples from each field replicate with 50 ml DI water and a 2 cm × 3.5 cm anion exchange membrane (AEM; Membranes International Inc., Ringwood, NJ) charged with 0.5 M $NaHCO_3$ for 4 h and extracted phosphate (PO_4^{3-}) adsorbed on the AEMs by shaking in 50 ml 0.5 M HC for 18 h. Adsorbed P was quantified using molybdate colorimetry (Murphy and Riley 1962) and a microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT).

Table 1 Ecosystem types, site locations, mean annual temperatures of the sample locations along with information source references

Ecosystem type	Location	Latitude	Longitude	MAT (°C)	References (supplemental)
Arid shrubland	Moab, UT, USA	36.83° N	109.41° W	12.5	Zelikova et al. (2012), Reed et al. (2012)
Mediterranean shrubland	Loma Ridge, CA, USA	33.73° N	117.70° W	17.0	Khalili et al. (2016)
Temperate grassland	MPG Ranch, Florence, MT, USA	44.66° N	114.01° W	7.0	Herget et al. (2015)
Temperate deciduous forest	Harvard Forest, Petersham, MA, USA	42.53° N	72.19° W	9.8	Abramoff and Finzi (2016)
Temperate coniferous forest	Lubrecht Experimental Forest, Greenough, MT, USA	46.9° N	113.46° W	7.0	Ganzlin et al. (2016)
Boreal forest	Bonanza Creek LTER, AK, USA	63.74° N	148.32° W	− 3.3	Burton et al. (2002)
Tropical rain forest	Barro Colorado Island, Panama	9.15° N	79.85° W	27.0	Baillie et al. (2007); Dieter et al. (2010)
Tropical rain forest	Piro Biological Station, Costa Rica	8.41° N	83.34° W	26.0	Weintraub et al. (2015)

Soil incubations

Historically, net P mineralization has been measured using soil incubations and a mass balance approach, similar to the characterization of net N mineralization (Jarvis et al. 1996). However, this method has been criticized for P because of rapid microbial P immobilization and geochemical sorption, leading to underestimations and/or misleading estimates of soil P turnover in many soils (Bünemann 2015). Other methods for measuring rates of net (and gross) P mineralization involve the use of radiolabeled P isotopic tracers, but costs and labor prohibit their implementation in a high-throughput manner (Bünemann 2015). Other non-isotopic methods used to measure gross P mineralization (Zou et al. 1992) have yielded substantial overestimations, and while modelling approaches parameterized by soil microbial and non-living P_o show promise in characterizing this elusive soil process (Bünemann 2015), they are still subject to the myriad challenges associated with measuring soil microbial P. Hence, soil P mineralization is notoriously difficult to measure, with the mass balance and isotopic approaches constituting opposite ends of a spectrum of measurement resolution.

Here, we adapted AEMs—frequently used to measure available, inorganic P in soils in situ (Cooperband and Logan 1994; Shaw and DeForest 2013) and in batch resin P extractions (Qian et al. 2008)—to capture PO_4^{3-} mineralized over the course of our incubations. AEMs are intended to simulate the anion exchange activities of roots by exchanging ions (here carbonate) adsorbed to their surface with higher affinity soil anions like PO_4^{3-}, driving diffusion gradients around the membrane's surface. A such, the P sorbed to AEMs exposed to soil is an approximation of labile, inorganic P availability over the timeframe of AEM contact with soil and can be converted into an estimate of soil P mineralization. The AEMs used in in this study were strong base anion exchange membranes composed of gel polystyrene cross linked with divinylbenzene (Membranes International, Inc., Ringwood, NJ). Each membrane had a total exchange capacity of 1.3 ± 0.1 meq g^{-1}, equivalent to a maximum of 20.6 mg PO_4^{3-} AEM^{-1} and must stay hydrated to function properly, nessecitating relatively high soil moisture.

To estimate soil P mineralization at each of our incubation temperatures (5, 15, 25, 35, and 45 °C), we incubated 10 g subsamples of field fresh soil from each site ($n = 3$) at field capacity in 50 ml centrifuge tubes with a 2 cm × 3.5 cm (14 cm^2 total surface area) AEM charged with sodium bicarbonate. Tubes were then placed in wide-mouth, pint size mason jars containing ~ 30 ml of DI water, sealed during incubation to maintain constant soil moisture. Three replicates from each site were incubated for 14 days at each incubation temperature. After 14 days, AEMs were removed, gently rinsed with DI water and extracted and analyzed as described previously for resin P analysis.

We limited our incubations to 14 days to limit exchange of PO_4^{3-} sorbed on the incubated AEMs with soil solution PO_4^{3-}. This bidirectional exchange of PO_4^{3-} can occur after the AEMs reach equilibrium with the soil solution (Meason and Idol 2008; Vandecar et al. 2011; Bünemann 2015) and is of particular concern in soils with high sorption capacity and low exchangeable PO_4^{3-}, as is common in highly weathered tropical soils. To combat this, we used the timeframe reported in Vandecar et al. (2011) for AEMs deployed in situ in strongly sorbing, low P soils to reach equilibrium with the soil solution, 14 days, for our incubations. Our goal in doing this was to prevent underestimation of the effects of temperature on P availability in tropical soils and to optimize the comparability of P mineralization data between soils.

Our method assumes that the PO_4^{3-} sorbed on incubated AEMs approximates P availability over the course of our 14 day incubations. Further, we assume that changes in soil exchangeable PO_4^{3-} concentrations are primarily a function of P mineralized by extracellular phosphatase activities and that movement of PO_4^{3-} is primarily unidirectional (from the soil solution to AEMs) over the 14-day incubation period. Accordingly, we assume that P recovered from AEMs incubated with soils at a range of temperatures reflects P made available over the course of the incubation, which is most likely to be mineralized P. We report both the total amount of AEM-sorbed P (i.e. mineralized P) over the course of our incubations as well as the estimated rate of P mineralization calculated by dividing this pool by the incubation timeframe (14 days). However, we acknowledge that our measurements generate an imperfect estimate of soil P mineralization. Because AEMs compete with soil microbes and the soil geochemical sink, our estimates likely underestimate this flux at field capacity, but we

contend that they are practically useful indices of P availability to plants and microbes, constituting a scalable, appropriate index of soil P mineralization at a level of resolution intermediate between the mass balance and isotopic approaches.

Soil extracellular enzyme analyses

Potential soil extracellular phosphomonoesterase (PMase) and phosphodiesterase (PDase) activities were measured as in Bell et al. (2013). Briefly, we made a 2.75 g of soil in 91 ml, 50 mM sodium acetate buffer slurry with each soil. The sodium acetate buffer was adjusted to site soil pH. We added aliquots of this slurry to 200 µM 4-methylumbelliferone (MUB)-P and bis-4-MUB-P substrates and 0, 2.5, 5, 10, 25, 50, and 100 µM MUB standards prepared in sodium acetate buffer and dispensed in 96 deep well plates. The deep well plates were incubated at 5, 15, 25, 35, or 45 °C for 24, 6, 3, 1.5, and 0.75 h, respectively. Incubation time reflects the time required at each temperature for the standard curves to develop without saturating. Incubated deep well plates were centrifuged for 3 min at 2900×g. Supernatant was then transferred to black, 96-well plates, and fluorescence was measured at 365 nm excitation and 450 nm emission using a microplate spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT, USA). Potential enzyme activities were calculated by fitting sample fluorescence at each temperature to its MUB standard curve and expressed as µmoles of hydrolyzed, fluorescently labeled substrate, 4-MUB-P and bis-4-MUB-P g soil^{-1} d^{-1} for PM and PD, respectively.

Reaction kinetics

Temperature response curves of net P mineralization and Ptase activities were fit according to macromolecular rate theory (MMRT) (Hobbs et al. 2013; Schipper et al. 2019). This modelling approach captures the thermodynamic behavior of soil macromolecules like enzymes, accounting for changes in their specific heat capacity (ΔC_P) with temperature and the resulting variation in temperature sensitivity with temperature (i.e. higher temperature sensitivity at lower temperatures). MMRT models the relationship between the rate of a biochemical process (k) and temperature (T) in K following Eq. 1,

$$\ln(k) - \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p (\ln T - \ln T_0)}{R} \quad (1)$$

where k_B is Boltzmann's constant, h is Planck's constant, H is enthalpy, S is entropy, and ‡ indicates the transition state. We used the method described in Heskel et al. (2016) and Liang et al. (2018) to fit this model to our data. In short, we fit a second-order polynomial to our net P mineralization and Ptase activity rate data (in µmol P g soil^{-1} day^{-1} and µmol bis-4-MUB P g soil^{-1} day^{-1}) plotted as a function of incubation temperature (T, in K) following Eq. 2,

$$\ln(k) = a + bT + cT^2 \quad \text{or} \quad \ln(k) = a + b(T - T_{(0)}) + c(T - T_0)^2 \quad (2)$$

using the Taylor expansion of Eq. 1 reported in Liang et al. (2018) (where $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$) and shown in Eq. 3,

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta G^{\ddagger}_{T_0}}{RT_0} + \left(\frac{1}{T_0} - \frac{\Delta H^{\ddagger}_{T_0}}{RT_0^2}\right)(T - T_0) + \left(\frac{\Delta C^{\ddagger}_p}{2RT_0^2}\right)(T - T_0)^2 \quad (3)$$

Such that $\Delta C^{\ddagger}_p, \Delta H^{\ddagger}_{T_0}$, and $\Delta G^{\ddagger}_{T_0}$ can be derived using Eq. 4.

$$a = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta G^{\ddagger}_{T_0}}{RT_0}, \quad b = \left(\frac{1}{T_0} - \frac{\Delta H^{\ddagger}_{T_0}}{RT_0^2}\right), \quad c = \left(\frac{\Delta C^{\ddagger}_p}{2RT_0^2}\right) \quad (4)$$

We calculated the temperature sensitivity of net P mineralization and Ptase (PMase and PDase) activities, we PMase, and PDase activites following Eq. 5.

$$Q_{10} = e\left(\frac{10\left(\Delta H^{\ddagger} - 5\Delta C^{\ddagger}_p\right)}{RT^2}\right) \quad (5)$$

Statistical analyses

Statistical analyses were conducted using R (R Core Team 2013) and figures were produced using the ggplot2 package (Wickham 2009). Data were tested for normality using the Shapiro–Wilk test and homoscedasticity using the Bartlett test. When data did not meet assumptions of normality required for parametric tests, values were natural log (ln) transformed. Significant differences between sites were tested for using paired T-tests, ANOVA and Tukey HSD tests ($P \leq 0.05$).

Results

Soil P availability (measured as AEM-sorbed P) increased with incubation temperature (Table 2). Parallel measured rates of P mineralization increased with incubation following MMRT-based kinetics (Fig. 1a). However, the standard error of the calculated change in heat capacity ($\Delta C_p^{\ddagger}$) of the underlying Ptase activities presumed responsible for P mineralization resulted in estimates of $\Delta C_p^{\ddagger}$ that included zero in five of our eight sites (arid shrubland, temperate deciduous forest, temperate coniferous forest, temperate grassland, and boreal forest). Further, the soil from Panamanian and Costa Rican tropical rainforests had slightly higher P availability at the lowest incubation temperature (5 °C) and were otherwise insensitive to temperature between 15–35 and 15–45 °C, respectively. This resulted in positive $\Delta C_p^{\ddagger}$ values, which are inconsistent with the thermodynamic theory underlying MMRT (Hobbs et al. 2013).

PMase and PDase activities also increased with temperature following MMRT-based kinetics as (Fig. 1b, c, respectively). Unlike the temperature response curves for P mineralization, the temperature dependence of $\Delta C_p^{\ddagger}$ of PMase and PDase was clear at most sites. The only exceptions to this were ($\Delta C_p^{\ddagger}$) caluclations with standard errors that included zero in the arid shrubland soils for both PMase and PDase and in the boreal forest soil for PMase. The activities of PMase and PDase were strongly correlated with one

Table 2 Initial soil pH, resin P concentrations, and total P sorbed to anion exchange membranes (AEM) incubated in soils at each incubation temperature (5 °C, 15 °C, 25 °C, 35 °C, and 45 °C)

Ecosystem type	pH	Initial Resin P (μg P g soil^{-1})	AEM-sorbed P (μg P g soil^{-1})				
			5 °C	15 °C	25 °C	35 °C	45 °C
Arid Shrubland	7.3 ± 0.1^{c}	1.56 ± 0.66^{c}	0.72 ± 0.16^{abc}	0.96 ± 0.24^{a}	1.32 ± 0.23^{a}	1.56 ± 0.45^{a}	2.48 ± 0.55^{ab}
Mediterranean shrubland	5.5 ± 0.1^{ab}	4.82 ± 0.17^{d}	3.06 ± 1.14^{c}	4.66 ± 0.73^{c}	10.8 ± 1.68 ^{cd}	13.7 ± 1.77^{c}	18.7 ± 3.64^{d}
Temperate grassland	5.8 ± 0.3^{a}	15.60 ± 5.0^{e}	5.61 ± 1.86^{d}	6.68 ± 1.34^{d}	11.3 ± 3.30^{d}	17.8 ± 6.60^{c}	22.1 ± 6.01^{d}
Temperate deciduous forest	3.5 ± 0.02^{d}	0.48 ± 0.17^{ab}	0.13 ± 0.11^{ab}	0.52 ± 0.04^{a}	0.97 ± 0.71^{a}	2.41 ± 1.73^{ab}	5.44 ± 2.56^{ab}
Temperate coniferous forest	5.3 ± 0.3^{ab}	5.04 ± 2.48^{d}	2.81 ± 0.98^{bc}	3.38 ± 0.98^{bc}	6.13 ± 0.64^{bc}	10.7 ± 3.97^{bc}	15.6 ± 3.00^{cd}
Boreal forest	4.4 ± 0.4^{c}	0.89 ± 0.38^{bc}	0.84 ± 0.35^{abc}	1.44 ± 0.64^{ab}	2.97 ± 0.73^{ab}	4.69 ± 1.12^{ab}	9.07 ± 0.65^{bc}
P tropical rainforest	5.4 ± 0.2^{ab}	0.29 ± 0.08^{a}	0.17 ± 0.03^{a}	0.14 ± 0.01^{a}	0.14 ± 0.02^{a}	0.25 ± 0.09^{a}	0.69 ± 0.18^{a}
CR tropical rainforest	5.0 ± 0.2^{b}	0.22 ± 0.08^{a}	0.29 ± 0.09^{a}	0.04 ± 0.01^{a}	0.07 ± 0.04^{a}	0.08 ± 0.02^{a}	1.17 ± 0.09^{b}

Values are means of analytical replicates (n = 3) ± standard deviation. Different letters indicate significant differences between sites, based on Tukey's HSD test ($P \leq 0.05$). P tropical rainforest is in Panama, and CR tropical rainforest is in Costa Rica (see Table 1)

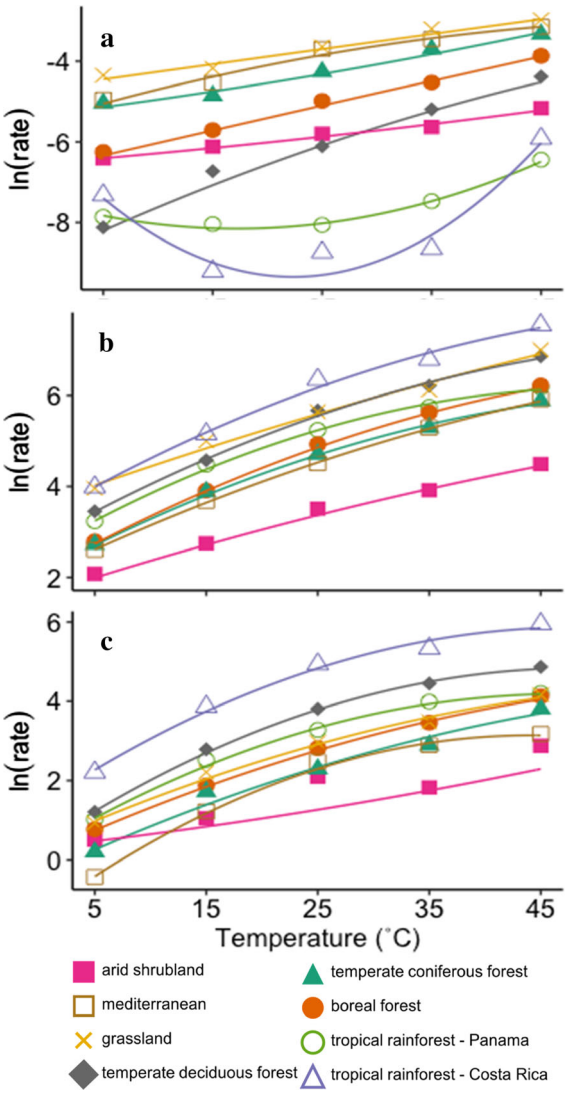

Fig. 1 The natural log of mineralized soil P, phosphomonoesterase (PMase) activities, and phosphodiesterase (PDase) acitivites plotted as a function of incubation temperature, with each point representing the mean rate by site at each temperature (°C) (■: arid shrubland, □: Mediterranean, ×: grassland, ◆: temperate deciduous forest, ▲: temperate coniferous forest, ●: boreal forest, ○: tropical rainforest-Panama, △: tropical rainforest-Costa Rica). Temperature response curves are fitted for each site according to MMRT-based kinetics

another across all sites ($R^2 = 0.89$, $P \leq 0.001$, Fig. S2). When data from all sites were analyzed together, there was no correlation between Ptase activity and mineralized P at any incubation temperature. However, for individual sites, P mineralization was significantly and positively correlated with PMase and PDase activities following power relationships (log–log linear regression) at all sites ($P < 0.01$) except for the two tropical rainforest sites, at which P mineralization increased exponentially with PMase and PDase activities ($P < 0.001$ and $P < 0.05$, respectively).

At all sites, the Q_{10} values of P mineralization, PMase, and PDase were significantly different from each other at all incubation temperatures. However, there was some variation in the directionality of these relationships among sites (Fig. 2). The Q_{10} of PDase was significantly higher than that of PMase ($P < 0.001$) except for the arid shrubland site, where the relationship was reversed ($P < 0.001$) (Fig. 2). Similarly, at all sites, the Q_{10} of P mineralization was significantly lower than those of both PMase and PDase at all site except for the temperate deciduous forest site, where the Q_{10} of P mineralization was intermediate between that of PMase and PDase. The Q_{10} values of PMase and PDase were significantly and positively correlated with one another at every site ($P < 0.001$) and incubation temperature ($P \leq 0.01$). Futher, the Q_{10} values of P mineralization were significantly and strongly correlated with both PMase and PDase activities ($P < 0.001$; $R^2 \geq 0.99$). The relationship between the Q_{10} of P mineralization and those of both PMase and PDase was positive at all sites except for the CR tropical rainforest site, where it was negative. However, there was no significant relationship between P mineralization and either enzyme at any incubation temperature across sites (Fig. 3).

Initial soil resin P spanned three orders of magnitude across all sites, from 0.22 ± 0.08 (CR tropical rainforest) to 15.6 ± 5.0 μg P g soil^{-1} (temperate grassland) (Table 3). Soil P mineralization was significantly and positively correlated with initial soil resin P at all temperatures. ($P \leq 0.001$; $R^2 = 0.65$, Fig. S3). Neither PMase nor PDase activities were correlated with initial resin P concentrations at any incubation temperature. The Q_{10} values of both PMase and PDase were negatively correlated with soil pH at all incubation temperatures ($P < 0.05$; Fig. S3). However, there was no significant relationship between the Q_{10} of Ptase activities and MAT or latitude at any incubation temperature. Further, there was no significant relationship between initial resin P and site latitude ($P = 0.18$), site MAT ($P = 0.11$), or soil pH ($P = 0.51$).

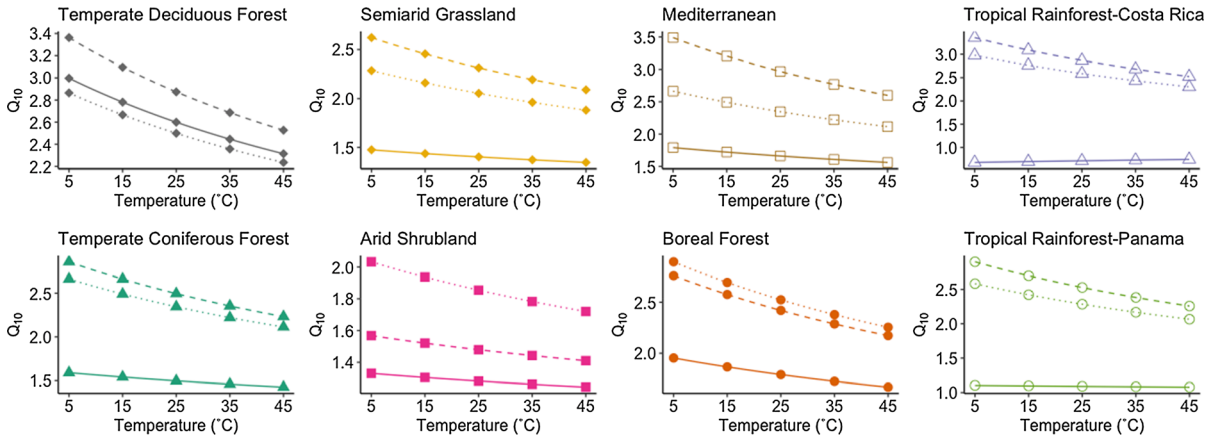

Fig. 2 The Q_{10} of P mineralization (solid line), phosphomonoesterase (PMase) activities (dotted line), and PDase activities (dashed line) plotted as a function of temperature for each of the eight ecosystem type representative sites

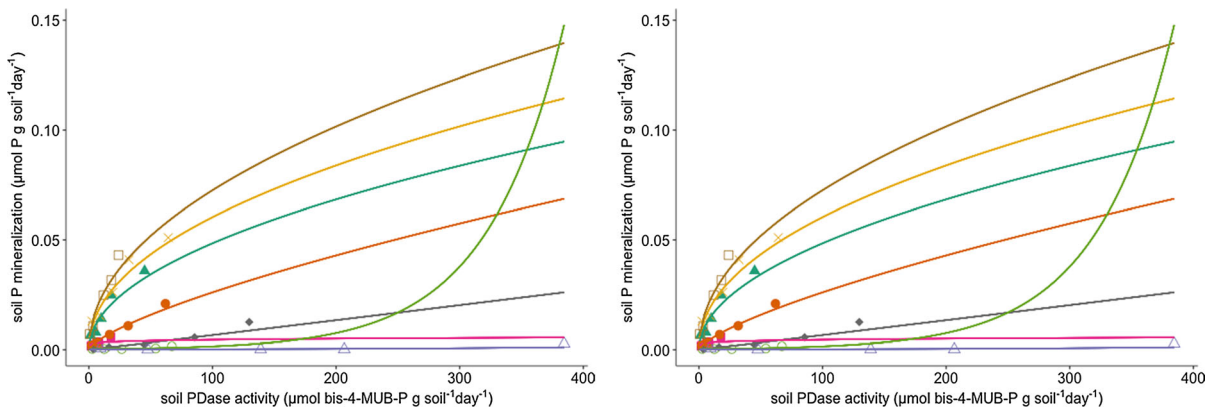

Fig. 3 Rates of soil potential net P mineralization by site (■: arid shrubland, □: Mediterranean, ✕: grassland, ◆: temperate deciduous forest, ▲: temperate coniferous forest, ●: boreal forest, ○: tropical rainforest-Panama, △: tropical rainforest-Costa Rica) plotted as a function potential phosphomonoesterase (PMase; **a**) and phosphodiesterase (PDase; **b**) activities

Discussion

Understanding how soil P availability responds to increasing temperature is critical to predicting the extent to which P availability may constrain future plant growth, yet the effects of temperature on P mineralization in soils are poorly understood. Resolving these effects is critical to estimate global C dynamics in ESMs and to our general understanding of the terrestrial P cycle. We hypothesized that soil warming would enhance soil P availability by accelerating rates of P_o hydrolysis via extracellular phosphatase enzymes, resulting in increased rates of soil P mineralization. Consistent with our hypothesis, both P availability and Ptase activities increased with temperature in every soil we measured (Figs. 1, 2). However, while these processes and their temperature senstitivities (as Q_{10}) were correlated at all sites, PM and PDases were significantly more sensitive to temperature than realized P mineralization in all sites but one, the temperate deciduous forest (Fig. 2). Overall, the responses of Ptase activities to temperature were generally consistent with enzyme kinetic theory, but realized rates of P mineralization—and therefore P availability—did not fit MMRT-based kinetics as well. Given the role of Ptases as the underlying mechanism of P mineralization, why might this be? We argue that the actual temperature response of P mineralization at each site represents the effects of a multitude of possible controls, of which Ptase

Table 3 The change in specific heat capacity ($\Delta C_p^{\ddagger}$) with temperature calculated using MMRT-based kinetics for soil P mineralization, phosphomonoesterase (PMase), and phosphodiesterase (PDase) activities for each of eight ecosystem type representative sites reported ± standard error

Ecosystem type	$\Delta C_p^{\ddagger}$		
	P mineralization	PMase activities	PDase activities
Arid Shrubland	0.22 ± 0.51	− 0.53 ± 0.87	0.44 ± 2.87
Mediterranean shrubland	3.18 ± 0.47	− 2.04 ± 0.46	− 2.64 ± 0.66
Temperate grassland	− 1.02 ± 1.79	− 1.56 ± 0.48	− 2.84 ± 0.54
Temperate deciduous forest	0.37 ± 0.67	− 1.58 ± 0.54	− 1.34 ± 0.77
Temperate coniferous forest	− 0.95 ± 0.51	− 1.05 ± 0.47	− 3.44 ± 0.72
Boreal forest	− 0.01 ± 0.70	− 0.46 ± 0.50	− 1.40 ± 0.61
P tropical rainforest	− 0.04 ± 0.76	− 1.51 ± 0.72	− 1.35 ± 0.64
CR tropical rainforest	9.57 ± 1.04	− 1.60 ± 0.45	− 2.86 ± 0.52

More information available about each site in Table 1

activity is only one. Below, we explore the relationship between soil Ptases and realized P mineralization in an attempt explain why the temperature responsiveness of Ptase activities may not directly translate to increases in soil P availability.

The relationship between Ptases and mineralized P in soils is one of plant and microbial *investment* in P_o acquisition enzymes, and the *return* on that investment as mineralized P (Allison et al. 2011; Fujita et al. 2017). Indeed, soil Ptase activity assays quantify this investment by measuring the amount of P_o hydrolyzed when substrate concentrations and accessibility are not limiting and pH is buffered (Allison et al. 2011; Fujita et al. 2017). Microbial investment in P_o acquisition (as Ptase pool size) varies as a function of microbial biomass and microbial P demand (Allison et al. 2011), irrespective of the temperature sensitivitiies of the Ptases produced. By contrast, P sorbed to AEMs during our incubations approximates the amount of labile, P_i liberated by the enzymatic hydrolysis of P_o under actual soil conditions. In natural soils, the abundance and accessibility of P_o substrate and pH are heterogeneous (Ettema and Wardle 2002), and covariation in any of these factors with MAT or latitude could partially explain Ptase activity and P mineralization.

Indeed, enzyme kinetic theory predicts that there is an overall negative relationship between MAT and the temperature sensitivity of soil extracellular enzyme activities (Allison et al. 2011; Wallenstein et al. 2011), and such variation has been identified for several enzymes (German et al. 2012). However, we found no relationship between Ptase activities nor their temperature sensitivities and MAT (or latitude) that would indicate such local climate adaptation of Ptases. This is inconsistent with previous work showing a positive correlation between acid Ptase activities and MAT globally (Margalef et al. 2017). One possible explanation for this is that acid phosphatases are broadly more temperature sensitive than alkaline phosphatases, given that we identified a negative correlation between the Q_{10} values of both PM and PDase and soil pH.

Regardless of microbial investment in P_o acquisition, increases in P mineralization and soil available P require a sufficient supply of P_o substrate. Yet, soil P_o varies both qualitatively (Turner et al. 2007; Turner and Engelbrecht 2010) and quantitatively (Yang and Post 2011) in field soils. As soils weather, P_o concentrations tend to increase, but they also make up a substantially larger fraction of the total soil P pool, increasing plant and microbial competition for P_o (Turner 2008). Thus, the most highly weathered soils tend to have the lowest concentrations of labile P_i (Yang and Post 2011). The most highly weathered (tropical rainforest) soils studied here had the lowest concentrations of initial resin P, followed closely by the temperate deciduous forest site. The temperate deciduous forest site soils are unglaciated and comparatively pedologically "young" in addition to

having a cooler climate with more pronounced themperature seasonaility. As a result, P_o substrate is likely much more abundant in the temperate deciduous forest site, allowing for more of the enzymatic capacity to increase P availability to be realized. In other words, substrate availability may play a commanding role in increasing the P availability return on microbial investment in Ptase enzymes.

Beyond the overall abundance of P_o compounds in soil, the chemistry of P_o in a given soil may also affect the efficiency of P_o mineralization. According to enzyme kinetic theory, enzymes that hydrolyze more complex substrates (such as PDases) should be more temperature sensitive than those that hydrolyze less complex substrates (such as PMases) (Allison et al. 2011; Wallenstein et al. 2011; German et al. 2012). Such simple P_o substrates tend to be less abundant in highly weathered soils (Turner et al. 2007) which could result in latitudinal variation in P_o chemistry and, by extension, sensitivity of P mineralization to warming. We found evidence supporting higher temperature sensitivities of PDases than PMases in all but one of our sites, the arid shrubland. This suggests that the mineralization of phosphate held in phosphodiester bonds is likely more sensitive to temperature than that of phosphate held in phosphomonoester bonds. However, we found no evidence that variation in pedogenic age, a proxy for P_o chemistry, contributes to variation in the efficiency of P mineralization with warming. Indeed, rates of P mineralization were least temperature sensitive in the sites with the most highly weathered soils, the tropical rainforests.

Regardless of the amount or types of P_o in a given soil, Ptases must also be able to *access* those substrates to hydrolyze them. Two major controls on this are soil moisture and soil minerology (Zimmerman and Ahn 2010). Phosphorus mineralization occurs in soil water and is therefore dependent on the availability of water to facilitate diffusion of Ptases, substrate, and hydrolyzed P. Thus, water availability is critically important to the P mineralization response to temperature (Margalef et al. 2017), as has been shown for N mineralization (Liu et al. 2016). However, evidence from other studies suggests that the effect of soil moisture on P mineralization may be similarly variable among, and even within ecosystem types (Zhou et al. 2013). In our experiment, we added water to the incubation vessels, alleviating any potential water limitation in the Ptase and P mineralization assays. In the seasonally dry soils investigated here (the Mediterranean and temperate grassland sites), we observed relatively high rates of Ptase activities, indicative of a reserve of Ptases in these soils collected during the dry season. However, we observed relatively low Ptase activities in the much drier arid shrubland site along with reversed temperature sentitivites of PMase and PDase. Finally, both P_o substrantes and Ptases react, variably, to mineral surfaces which can affect the accessibility of P_o substrates to Ptases and therefore the rate of P mineralization (Zimmerman and Ahn 2010).

Together, our results show an overall positive response of P mineralization to warming, but indicate that the temperature sensitivity of Ptase activities is a poor predictor of the temperature sensitivity of P mineralization. Thus, using Ptase enzyme temperature sensitivities as a proxy for P mineralization may overestimate the temperature sensitivity of P mineralization, and thus overestimate future P supply under most conditions. Instead, we argue that predicting the temperature sensitivity of soil P mineralization requires an understanding of botgh Ptase activities and the factors that may constrain its efficiency, primarily P_o availability. Further, given significant differences in PMase and PDase, the incorporation of finer-resolution, biologically-based soil P_o pools may be more useful than total? P pools (DeLuca et al. 2015). Indeed, our results suggest that something akin to the substrate supply hypothesis, whereby soils with the most abundant C substrate have the highest maximum decomposition rates and optimum decomposition temperatures (Richardson et al. 2012), may apply to soil P mineralization.

The effects of climate warming on nutrient supply pathways are likely to influence terrestrial ecosystem responses to global change broadly, and will almost certainly strongly regulate the magnitude of the CO_2 fertilization effect on NPP (Brovkin and Goll 2015; Wieder et al. 2015). Yet, the response of nutrient mineralization processes to warming remains a source of considerable uncertainty in predicting future nutrient supplies. Our results suggest that like N mineralization (Liu et al. 2016; Rustad et al. 2001), rates of P mineralization are sensitive to temperature. Thus, climate warming could enhance soil P availability across a broad range of natural ecosystems. Clearly, our short-term incubation experiments did not

accurately simulate the effects of a predicted global increase in MAT of 3–5 °C (Wieder et al. 2015). However, given the increases in P mineralization we observed, we suggest that climate warming has the potential to meet some portion—perhaps a significant portion—of the increasing P demand to support future NPP predicted by Wieder et al. (2015). We suggest that future work should extend this research to field warming studies in a range of ecosystem types to establish the relationship between temperature, P mineralization, and Ptase activities in the field and clarify the role of specific constraints to P_o mieralization.

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Data availability All data will be published in the EarthChem data repository upon manuscript acceptance.

Code availability R code used for data analysis will be made available upon acceptance on Github.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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