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Kinetic parameters of phosphatase: A quantitative synthesis

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ABSTRACT

Phosphatases play an important role in mineralization of organic phosphorus, soil phosphorus availability and global phosphorus cycling. Release of phosphorus in different ecosystems is important for plant growth and microbial function, and may be simulated by modeling organic phosphate mineralization. The half-saturation constant (K_m) and the maximum enzyme activity (V_{max}) in the Michaelis–Menten equation are the two important kinetic parameters in these models, but their values have not been systematically investigated. In this study, we compiled a database of kinetic parameters of phosphatase from 139 publications, estimated the means, variations and distributions of the kinetic parameters, and tested the differences in kinetic parameters of phosphatases of different types, origins and under different incubation conditions. We also analyzed the activation energy (E_a), temperature sensitivity (Q_{10}), optimum pH (pH_{opt}) and sensitivity of pH (pH_{sen}) of phosphatase activity. Our results indicated that: 1) Both V_{max} and K_m were log-normal distributed with large variations; 2) There was no significant difference in K_m between the acid or alkaline phosphatases, but a significantly higher V_{max} for acid phosphatases was found compared with alkaline phosphatases; 3) K_m and V_{max} varied with the origins of enzymes and under different incubation conditions. Plant originated enzymes had the highest V_{max} while soil originated enzymes had the lowest V_{max}. Larger variation in V_{max} was found among the incubation times than among the incubation temperatures; 4) The mean values of E_a for acid and alkaline phosphatases were 36.30 and 23.61 kJ mol^{-1}, respectively, with an overall mean of 34.40 kJ mol^{-1}. The mean value of estimated pH_{opt} for acid phosphatase was 5.2 while that for alkaline phosphatase was 9.5. The information generated in this study will be useful for phosphorus mineralization modeling and uncertainty analysis.

1. Introduction

Phosphorus (P) is an essential macronutrient utilized by all organisms for energy transport and growth (Krämer and Green, 2000). It is involved in many critical biological processes, such as energy metabolism, synthesis of nucleic acids and membranes, and photosynthesis (Raghothama, 1999; Vance et al., 2003). Globally, P is still a major nutrient that limits crop production and plant productivity in many different ecosystems, especially in highly weathered, acidic or calcareous soils (Holford, 1997; Sánchez and Salinas, 1981; Chapin and Kedrowski, 1983; Krämer and Green, 2000). P also interacts with other essential elements such as carbon (C) and nitrogen (N) in regulating biological processes, and the ratio of C:N:P is considered as an important indicator in estimating C and nutrient fluxes in global circulation models.

Availability of P may be regulated by many factors. In natural ecosystems, P may originate from the mineralization of plant litter, algae, soil organic matter and sediments (Eivazi and Tabatabai, 1977; Bae and Barton, 1989; Yadav and Yadav, 1996; Joner et al., 2000; Vance et al., 2003). P exists in either inorganic (*Pi*) or organic (*Po*) forms in the soil, where *Po* and *Pi* are operationally defined based on the Hedley fractionation (Cross and Schlesinger, 1995). *Po* is believed to be biologically assimilable while *Pi* is believed to be geochemically bound. Plants can only utilize *Po* after hydrolysis (Adams and Pate, 1992; Tarafdar and Claassen, 1988). Phosphatases include a group of enzymes that can hydrolyze the ester-phosphate bonds in soil organic P, which releases the phosphate into soil solution for uptake by nearby roots or microbes (Tarafdar and Claassen, 1988; Pant and Warman, 2000; Duff et al., 2006). There are two major extracellular enzymes of phosphatase: acid and alkaline phosphatases, classified according to their most effective pH. Acid phosphatase activity, in particular, may provide a large portion of *Pi* for plants (Harrison and Pearce, 1979; Kroehler and Linkins, 1988; Moorhead and Reynolds, 1993). Both of

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them significantly contribute to the *Pi* release in the soils and nutrient cycling on the earth.

Phosphatase activity can be detected using a quantitative measurement of hydrolysis of a substrate, either measured as disappearance of the substrate or as formation of one of the two resulting products. Since its introduction decades ago (Tabatabai and Bremner, 1969), the use of p-nitrophenyl phosphate (*p*NPP) as a substrate in quantitative measurements of endogenous soil phosphatase and extracellular phosphatase of plants and microorganisms has dominated due to its convenience (Joner et al., 2000). This activity assay method measures activities of extracellular enzymes, which are released by microorganisms to initially cleave organic matter into smaller molecules (Tabatabai and Bremner, 1969; Williams et al., 1973; Juma and Tabatabai, 1988; Coolen and Overmann, 2000; Waldrop et al., 2004; Wallenstein and Weintraub, 2008; Henry, 2012; Burns et al., 2013). In the case of substrate *p*NPP, p-nitrophenol (*p*NP) formed after hydrolysis will be subsequently extracted with chemical such as sodium hydroxide (NaOH) and then measured spectrophotometrically. From such measurements, the affinity and potential rate of extracellular hydrolysis of biopolymers can be inferred, using the Michaelis–Menten (M–M) kinetics (Michaelis and Menten, 1913; Tabatabai and Bremner, 1971; Williams et al., 1973; Juma and Tabatabai, 1988; Grant et al., 1993; Nannipieri and Gianfreda, 1998; Manzoni and Porporato, 2009; Allison et al., 2010; Wang and Post, 2013; Wang et al., 2012, 2013):

$$V = V_{\max}\frac{S}{K_m + S} \quad (1)$$

where V is the enzyme reaction rate; $V_{\max}$ and K_m are the maximum enzyme activity and the half-saturation constant, respectively; and S is the concentration of substrate. Both K_m and $V_{\max}$ can be derived using a series of substrate concentrations at certain pH and temperature. Enzymatic activity usually varies with temperature and pH and enzymes often have different optimum temperature and pH. The response of enzyme activity to temperature change can be described using the Arrhenius equation in which the activation energy (E_a) is a key parameter (Johnston, 1975; Feng et al., 1990; McClaugherty and Linkins, 1990; Scrutton et al., 2001; Calsavara et al., 2001). These model parameters and the influences of temperature and pH can be built into mineralization models to simulate P release and cycling.

Many studies have been conducted on phosphatase, including kinetics, regulation of enzymatic activity by temperature and pH, influence by substrate concentrations, inhibitors, and effects of many different treatments (Verchot and Borelli, 2005). But there is a lack of syntheses of the kinetic parameters which is necessary to enable modeling. Although current phosphorus models mostly estimate mineralized phosphorus by either converting from mineralized carbon or nitrogen using C:P or N:P ratio, or through a rate coefficient for mineralization (Jones et al., 1984; Treseder and Vitousek, 2001; Wang et al., 2010; Henry, 2012; Runyan and D'Odorico, 2012), some newly developed ecosystem models tend to consider the kinetics of enzyme activity and include enzyme pools in the simulation (Davidson et al., 2012; Moorhead et al., 2012; Sinsabaugh and Shah, 2012; Wang et al., 2013). For example, Sinsabaugh and Shah (2012) proposed a biogeochemical equilibrium model that combines the kinetics of enzyme activity and community growth under different resource limitations, based on metabolic and ecological stoichiometry theory. They suggest that phosphorus, rather than nitrogen, might be a constraint on microbial metabolism and call for better estimates of enzyme related model parameters and their variations.

The objective of this study was to document enzymatic parameters of phosphatase through a literature research and data synthesis. We have compiled a database of kinetic parameters for phosphatases and analyzed the means, variations and distributions. The influences of enzymatic activities of phosphatase by temperature and pH were also investigated. The database and information obtained in this study will be useful for enzyme-driven soil organic/sediment decomposition models to simulate nutrient release and nutrient cycling, and has important implications for plant growth and nutrient dynamics and cycling.

2. Materials and methods

2.1. Literature review and data collection

We compiled a database of kinetic properties of phosphatases including acid phosphatases and alkaline phosphatases from the literature up to July 2012. We searched the online databases (i.e. Web of Science and ScienceDirect) using the keywords kinetic, phosphatase, K_m and $V_{\max}$. We included papers that reported both the half-saturation constant (K_m) and maximum enzyme activity ($V_{\max}$). For a small number of papers that the K_m and $V_{\max}$ were reported in figures, we manually digitalized them. If multiple values were reported in one paper under different treatments, we recorded all values with the treatment conditions. In total, we recorded 930 pairs of K_m and $V_{\max}$ and the corresponding experimental conditions (i.e. origin of enzyme, type of enzyme, substrate, maximum substrate concentration ($S_{\max}$), buffer, incubation temperature, pH and parameter estimation method) from 139 publications (Table 1).

2.2. Enzymatic activity assay and kinetic analysis

Several different methods were used in the literature to measure enzymatic activity (Joner et al., 2000), including a histochemical method of precipitating a Fast Blue RR salt with P from alpha-naphthyl acid phosphate to indicate metabolically active cells or cell components (Tisserant et al., 1993), a qualitative visualization employing phenolphthalein phosphate in agar plates to demonstrate extracellular phosphatase activity (Trolldenier, 1992), and a quantitative measurement of hydrolysis of a substrate, either measured as disappearance of the substrate or as formation of one of the two resulting products. But since Tabatabai and Bremner (1969) proposed to use *p*NPP as a substrate instead of the PP (phenyl phosphate), most of the studies adopted this method.

Conditions of the assay must be controlled with respect to temperature, duration, pH, and ionic strength of the solution (Tabatabai, 1994; Verchot and Borelli, 2005). Some investigators used different incubation conditions such as incubation temperatures, times, buffers, or used a slightly different wavelength from 410 nm for color absorption measurements. For kinetic analysis, enzymatic activity was measured under a series of different substrate concentrations. The starting substrate concentration was close to 0 mM, and the high level of substrate concentration varied from close to 1 mM to more than 500 mM, with majority between 5 mM and 50 mM.

Depending on the pH of the incubation medium, one can distinguish acid phosphatase activity from alkaline phosphatase activity, measured respectively at pH around 5 and 8 (Bae and Barton, 1989; van Aarle and Plassard, 2010).

2.3. Estimation of kinetic parameters using Michaelis–Menten (M–M) model

As important kinetic parameters, $V_{\max}$ and K_m have been extensively investigated to characterize the enzyme-driven

Table 1
Basic information of the database. Sample size, mean maximum enzyme activity (V_{max}) and half-saturation constant (K_m) (±standard error) among enzyme types, incubation conditions, and estimation methods. Same letters in one category indicate not significance at $\alpha = 0.05$ level.

	Sample size (n)	Log(K_m) (mM)	Log(V_{max}) (μmol *p*NP g^{-1} h^{-1})
Type			
Acid	532	0.76 ± 0.10a	5.32 ± 0.27a
Alkaline	246	1.13 ± 0.17a	3.67 ± 0.23b
Origin			
Plants	217	0.38 ± 0.16ef	8.47 ± 0.36a
Soils	333	0.87 ± 0.12de	2.61 ± 0.21c
Wetlands	36	−1.04 ± 0.33g	2.88 ± 0.36c
Lakes	61	0.88 ± 0.36de	5.45 ± 0.12b
Bacteria	29	1.51 ± 0.39cd	6.27 ± 0.99b
Fungi	21	−0.37 ± 0.41fg	5.10 ± 0.88b
Moss	49	4.40 ± 0.14b	3.29 ± 0.17c
Sludge	32	5.75 ± 0.09a	5.32 ± 0.06b
Others	36	1.92 ± 0.35c	3.36 ± 0.43c
Incubation temperature (°C)			
37	398	1.04 ± 0.12b	5.18 ± 0.25a
30	107	2.20 ± 0.31a	5.24 ± 0.34a
25	75	1.19 ± 0.30b	5.18 ± 0.52a
20	150	0.22 ± 0.21c	3.03 ± 0.39b
Other	116	1.34 ± 0.24b	4.68 ± 0.42a
Incubation time (min)			
10	54	−1.26 ± 0.19e	9.86 ± 0.68a
20	34	0.92 ± 0.39bc	8.38 ± 0.94a
30	74	2.08 ± 0.29a	3.35 ± 0.38c
60	373	1.66 ± 0.14ab	3.91 ± 0.15bc
90	34	−0.35 ± 0.34d	5.39 ± 0.36b
Others	277	0.73 ± 0.14c	4.64 ± 0.37bc
Substrate			
*p*NPP	706	1.20 ± 0.18a	5.11 ± 0.52a
Others	140	0.58 ± 0.10b	2.77 ± 0.16b
Maximum substrate concentration (mM)			
$S_{max} \leq 5$	335	1.38 ± 0.18ab	5.79 ± 0.21a
$5 < S_{max} \leq 15$	133	0.44 ± 0.21c	5.82 ± 0.38a
$15 < S_{max} \leq 35$	110	1.27 ± 0.12b	2.21 ± 0.27b
$S_{max} > 35$	148	1.92 ± 0.14a	2.68 ± 0.28b
Buffer			
Sodium	100	−1.06 ± 0.14b	8.71 ± 0.48a
Tris–HCl	170	1.33 ± 0.23a	5.68 ± 0.30b
MUB	109	1.62 ± 0.11a	1.61 ± 0.17d
Others	255	1.38 ± 0.18a	4.74 ± 0.25c
Stopper			
NaOH	416	0.78 ± 0.14a	5.97 ± 0.21a
Others	430	1.39 ± 0.11b	3.40 ± 0.24b
A410			
410 nm	149	1.94 ± 0.23a	5.13 ± 0.27a
Others	697	0.91 ± 0.10b	4.66 ± 0.19b

reactions using the M–M model as a hyperbolic function in Eq. (1) (Williams et al., 1973; Juma and Tabatabai, 1988; Nannipieri and Gianfreda, 1998; Wallenstein et al., 2011). To estimate V_{max} and K_m, different analytic methods have been employed in the literature. Most of the studies adopted the Lineweaver–Burk (LB) transformation which convert V and S into $1/V$ and $1/S$, and develop a linear regression equation of $1/V$ and $1/S$ to estimate V_{max} and K_m in Eq. (1) (Han and Srinivasan, 1969). The other transformation methods included Hanes–Wolf (HW), Eadie–Hofstee (EH), and Eisenthal & Cornish-Bowden and Wilkinson (Gerritse and Van Dijk, 1978; Cornish-Bowden and Cornish-Bowden, 1979; Vincent et al., 1992; Margon and Fornasier, 2008; Cabello-Díaz et al., 2012). Some studies have compared the linearization methods and found that the HW and EH method are superior to the LB (Atkins and Nimmo, 1975; Dowd and Riggs, 1965). However, LB is still widely used in the literature. Some other studies also used the nonlinear regression methods to directly estimate V_{max} and K_m in Eq. (1) (Firsching and Claassen, 1996; Zhang et al., 2009).

2.4. Response functions of enzymatic activity to temperature and pH

The kinetic properties of enzymes were usually measured under specific temperature and pH conditions. The influences of temperature and pH on the enzyme activity have been examined in many studies. Following Wang et al. (2012), the influences of temperature and pH on enzyme activity can be described as:

$$V = V_0 \cdot f(T) \cdot f(\mathrm{pH}) \tag{2}$$

where V is the enzyme activity at temperature (T) and pH; V_0 is the enzyme activity at a reference temperature (T_{ref}) and optimum pH (pH_{opt}).

The influence of temperature can be calculated using the Arrhenius equation (Maier et al., 1955; Eivazi and Tabatabai, 1977; Feng et al., 1990; Rao et al., 2000; Wallenstein et al., 2011):

$$f(T) = \exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \tag{3-1}$$

or

$$f(T) = V_a \exp\left[-\frac{E_a}{RT}\right] \tag{3-2}$$

where T and T_{ref} are the incubation temperature and reference temperature (K), respectively; E_a is the energy of activation (kJ mol^{-1}); $R = 8.314$ J mol^{-1} K^{-1}, is the universal gas constant; and V_a is the basal enzyme activity.

In many enzyme-driven biological processes, Q_{10} is widely used to express the temperature sensitivity (Davidson et al., 2006; Ise and Moorcroft, 2006):

$$f(T) = Q_{10}^{\frac{T - T_{ref}}{10}} \tag{4}$$

where Q_{10} is the factor by which enzyme activity is multiplied when temperature increases by 10 °C. Combining Eqs. (3) and (4), we can find the relationship between Q_{10} and E_a:

$$Q_{10} = \exp\left[\frac{E_a}{R \cdot T_{ref}} \cdot \frac{10}{T}\right] \tag{5}$$

The effect of temperature on enzyme activity V was often presented by data plots in the publications or reported as the activation energy E_a. If the data were reported in plots, we manually digitalized the data and estimated E_a using Eqs. (3-1) and (3-2). Q_{10} was calculated from E_a using Eq. (5). We calculated the means and variations of E_a and Q_{10}.

The response function of pH can be expressed by an exponential–quadratic function (Reth et al., 2005; Wang et al., 2012):

$$f(\mathrm{pH}) = \exp\left[-\left(\frac{\mathrm{pH} - pH_{opt}}{pH_{sen}}\right)^2\right] \tag{6}$$

where pH_{opt} is the optimum pH that gives the maximum reaction rate; and pH_{sen} refers to the sensitivity of the reaction rate to deviation from pH_{opt}.

Most studies reported the response patterns of enzyme activity V on pH using data plots. Using the method proposed by Wang et al. (2012), we estimated pH_{opt} and pH_{sen} using Eq. (6) through curve fitting of the pH response data. We redrew the data points via normalizing the V by the largest observed V. Thus the normalized V

values fell into the range between 0 and 1. Both pH_{sen} and pH_{opt} were estimated from the curve fitting. The means and variations of pH_{sen} and pH_{opt} for both acid and alkaline phosphatase were calculated.

2.5. Data analysis of kinetic parameters

To compare V_{max} from various experiments, we converted V_{max} from different units reported in the literature into the same units (μmol *p*NP g^{-1} h^{-1} or μmol *p*NP l^{-1} h^{-1}). K_m values were expressed in mM (mmol l^{-1}) in the studies. Majority of the studies used 37 °C as incubation temperature and conducted kinetic analysis at the pH close to optimal pH of enzymes. Since some studies collected enzymes in the water from oceans, freshwater systems, and marshes and reported the V_{max} in different units, we first separated the whole dataset into two groups: enzymes usually existing in the solution and enzymes collected in the soils and sediments. Following Wang et al. (2012), we also converted V_{max} to a reference temperature (set to 37 °C) and optimum pH (set 5.2 for acid phosphatases and 9.5 for alkaline phosphatases as estimated above) using Eqs. (2), (3) and (6). We calculated descriptive statistics such as mean, standard deviation for each group. Since we focused on terrestrial ecosystems in this study, we excluded the enzymes in the solution in further analyses. We tested whether origin, type and incubation methods had significant influences on V_{max} and K_m.

Statistical analyses of parameters were conducted using SAS software (Hui and Jiang, 1996; SAS Inc. Cary, NC USA). We calculated descriptive statistics of parameters such as mean, median, standard deviation, and coefficient of variation (CV). The original distributions of K_m and V_{max} seemed to be log-normal distributions. We implemented logarithmic data transformations to stabilize the variances (Bland and Altman, 1996; Wang et al., 2012). The significance of difference in kinetic parameters between the original of enzymes, type of enzymes (acid and alkaline), incubation temperature, time, buffer, substrate, maximum substrate concentration, stopper, and A410 were tested by one-way ANOVA (Hui and Jiang, 1996). The differences in kinetic parameters of various treatments were tested by the Fisher's least significant difference (LSD) at a significance level of $\alpha = 0.05$. The relationship between V_{max} and K_m was derived using a linear regression analysis for all data and for different sources using the categories in Table 1.

3. Results

3.1. Distributions of maximum enzyme activity (V_{max}) and half-saturation constant (K_m)

3.1.1. Maximum enzyme activity (V_{max})

We separated the whole database into enzymes collected from water and those from soils/sediments. Of total 935 pairs of kinetic parameters, 157 were from water and 778 observations of kinetic parameters for phosphatases from the soils/sediments. V_{max} for enzymes from both soils/sediments, and water showed great variability spanning several orders of magnitude from 10^{-3} to 10^{8} μmol *p*NP g^{-1} h^{-1} or 10^{-3} to 10^{4} μmol *p*NP ml^{-1} h^{-1}, respectively. The mean values of V_{max} were 2.05×10^{6} μmol *p*NP g^{-1} h^{-1} and 859.8 μmol *p*NP ml^{-1} h^{-1} with the coefficients of variation (CV) of V_{max} being 1226% and 245% for soils/sediments (Fig. 1a) and water, respectively. We used a logarithmic-transformation on V_{max} and fit log(V_{max}) with a normal distribution with $\mu = 4.73$ and $\sigma = 4.68$ (Fig. 1b) for phosphatases from the soils/sediments. The significance of difference was tested in terms of log(V_{max}) instead of V_{max}. Since enzymes in soils/sediments are more closely related to nutrient availability and P cycling, we only performed the further tests on enzymes measured in soils/sediments.

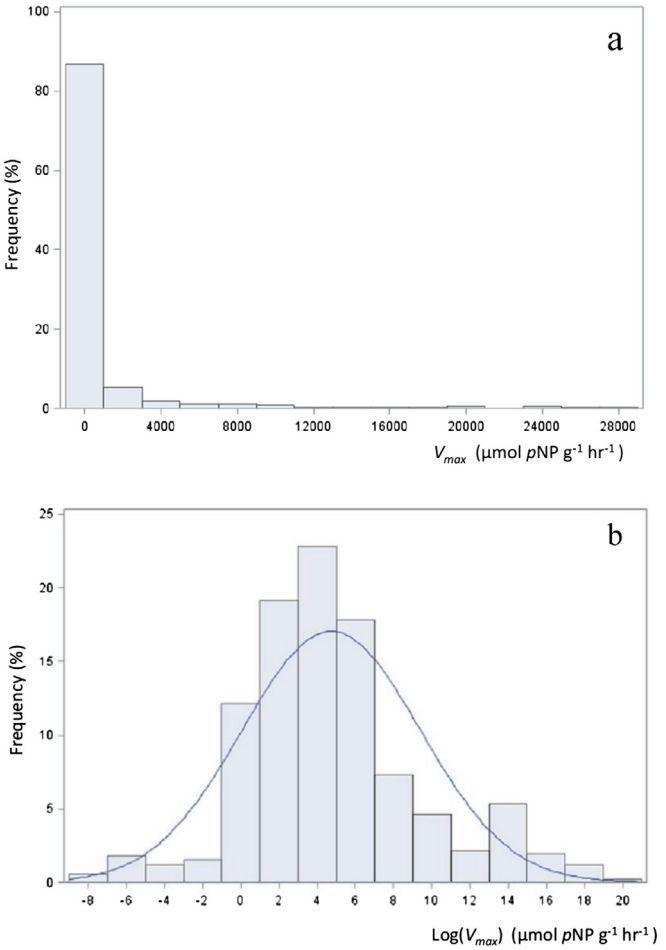

Fig. 1. Histograms of the maximum enzyme activity (V_{max}) for phosphatases in soils/sediments. a) Original data. Note not all data are shown. b) Log-transformed data. The line is fitted with a normal distribution of $\mu = 4.73$ and $\sigma = 4.68$.

3.1.2. Half-saturation constant (K_m)

K_m also spanned several orders of magnitude from 10^{-3} to 10^{3} mM for enzymes collected from soil/sediments (Fig. 2a) and 10^{-3} to 10 mM for those from water. A logarithmic-transformation of K_m showed a better fit for normal distribution with $\mu = 1.09$ and $\sigma = 2.60$ for phosphatases from the soils/sediments (Fig. 2b). Log(K_m) was also used for further statistical analysis.

3.2. Effects of incubation conditions on maximum enzyme activity (V_{max}) and half-saturation constant (K_m)

3.2.1. Maximum enzyme activity (V_{max})

We tested whether the origin of the enzymes (from plant, soil, etc), type of enzyme (acid or alkaline), and/or incubation conditions influenced the log(V_{max}) and log(K_m). The ANOVA tests showed that there were significant differences in log(V_{max}) between enzymes from different sources, types, incubation temperatures, durations of incubation time, substrates, buffers, enzyme activity stopper used, and among color absorption wavelengths (Table 1). The LSD test on log(V_{max}) indicated that the enzyme originated from plants had significantly higher values than those from bacteria, fungi and others (Table 1). Log(V_{max}) from soils was the lowest, but did not

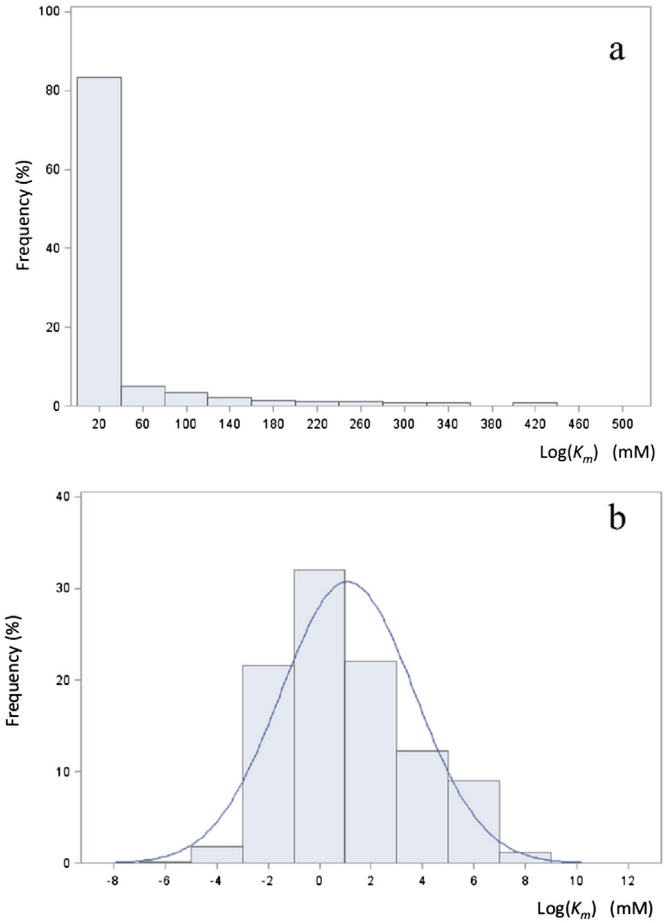

Fig. 2. Histogram of the half-saturation constant (K_m) for phosphatases in soils/sediments. a) Original data. Note not all data are shown. b) Log-transformed data. The line is fitted with a normal distribution of $\mu = 1.09$ and $\sigma = 2.60$.

differ significantly from those from wetland and moss. Acid phosphatase had significantly higher log(V_{max}) than alkaline phosphatase. Among different incubation temperature, only 20 °C showed significant lower log(V_{max}) than other temperature (25, 30, and 37 °C). Incubation for 10 and 20 min showed the highest log(V_{max}), significantly higher than other times. Incubation time of 30 min had the lowest log(V_{max}), but did not differ significantly from 60 min. Using *p*NPP as substrate had higher log(V_{max}) than other substrates. Maximum substrate concentration also significantly influenced log(V_{max}) with higher log(V_{max}) measured at lower maximum substrate concentrations. Using sodium as buffer produced the highest log(V_{max}) and MUB generated the lowest value. Log(V_{max}) was higher if NaOH was used as stopper than others.

3.2.2. Half-saturation constant (K_m)

The ANOVA tests showed that there were significant differences in log(K_m) between enzymes from different sources, but no difference between acid and alkaline phosphatases. We also found significant differences among incubation temperatures, durations, substrates, maximum substrate concentrations, buffers, stoppers, and A410 light sources. The multiple comparison test showed the mean log(K_m) was significantly higher in sludge than other sources. Incubation temperature of 30 °C had the highest log(K_m) and 20 °C had the lowest log(K_m). Incubation time of 30 min and 50 min had the highest log(K_m) and incubation time of 10 min and 90 min had the lowest log(K_m). Incubation using the substrate *p*NPP had lower log(K_m) than other substrates. Maximum substrate concentration

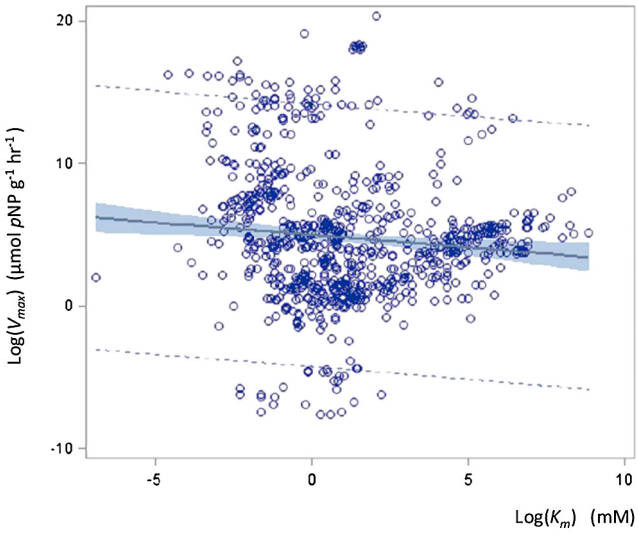

Fig. 3. Linear regression of the maximum enzyme activity [Log(V_{max})] and the half-saturation constant [Log(K_m)]. Log(V_{max}) = 4.941 − 0.176*Log(K_m), $r^2 = 0.01$, $p = 0.005$, $n = 810$.

influenced the estimation of log(K_m), and concentrations between 5 and 15 mM produced much lower log(K_m) than other concentrations. Using the sodium as buffer had the lowest log(K_m) than others.

3.3. Relationship between V_{max} and K_m

We tested whether the two model parameters were correlated. Using the original V_{max} and K_m, we did not find any significant relationship. When converted to the logarithmic units, log(V_{max}) and log(K_m) was significantly correlated ($p = 0.005$; Fig. 3). However, the coefficient of determination (r^2) was only 0.01, indicating a very weak linear relationship. We also developed the regression models of log(V_{max}) and log(K_m) for different sources using the categories in Table 1. Among 36 regression models developed, 21 were significant ($p < 0.05$). 25 models had r^2 smaller than 0.10. Only four r^2 were larger than 0.30 and one r^2 was larger than 0.50. Thus, the relationship between log(V_{max}) and log(K_m) was either not significant or weak.

3.4. Activation energy and temperature sensitivity

The mean values of E_a for acid and alkaline phosphatases were 36.30, 23.61 kJ mol^{-1}, respectively, with an overall mean of 34.40 kJ mol^{-1} (Table 2). The corresponding Q_{10} (base temperature = 20 °C) were 1.77, 1.42 and 1.72 for acid, alkaline phosphatases and overall. Both E_a and Q_{10} were higher for acid phosphatase than those for alkaline phosphatase.

3.5. Optimum pH, sensitivity of pH, and response function

The response function of pH was well expressed using the exponential-quadratic function. The coefficient of determination was mostly larger than 0.8. The mean value of estimated pH_{opt} for acid phosphatase was 5.2 while that for alkaline phosphatase was 9.5 (Table 3). The mean pH_{sen} was 1.8 for acid phosphatase and 2.6 for alkaline phosphatase. Both values were significantly higher for the alkaline phosphatase than the acid phosphatase.

Table 2
Activation energy (E_a) and temperature sensitivity (Q_{10}, base temperature = 20 °C) for phosphatases.

Enzyme	E_a (kJ mol^{-1})	Q_{10}
Acid	36.30 ± 2.80 (87)[a]	1.77 ± 0.13 (42)
Alkaline	23.61 ± 5.48 (15)	1.42 ± 0.12 (15)
All	34.40 ± 2.55 (102)	1.72 ± 1.16 (102)

[a] Mean ± standard error (sample size).

4. Discussion

By analyzing a kinetic parameter database for phosphatases compiled from the literature, we demonstrated that both V_{max} and K_m were log-normal distributed. Significant differences in V_{max} were found between acid and alkaline phosphatases and among phosphatases of different origins (e.g., plants, soils, etc.), and under different incubation conditions. Mean values of V_{max} and K_m of enzymes of different types and origins were estimated. The effects of temperature and pH on enzyme activity for both acid and alkaline phosphatases were also provided. To our knowledge, this study provides the first systematic synthesis on phosphatase kinetic parameters. The information provided in this study is useful in several aspects. First, it provides basic statistical description of V_{max} and K_m of phosphatase, an important enzyme in organic phosphorus mineralization. Second, the mean values of activation energy (E_a), Q_{10}, optimum and sensitivity of pH for acid and alkaline phosphatases provide a better understanding of phosphatase responses to temperature and pH. Lastly, modeling phosphorus availability and transformation is an important task in agriculture and global change ecology. The values generated in this study will be useful for modeling and uncertainty analysis of phosphorus cycling.

4.1. Differences in kinetic parameters between acid and alkaline phosphatases and across enzyme origins

We found significant difference in log(V_{max}) but no difference in log(K_m) between acid and alkaline phosphatases. Both acid and alkaline enzymes had similar affinity for their substrates, but acid phosphatases had a significantly higher log(V_{max}) (44.9%), and mineralize organic phosphorus at a higher rate compared to alkaline phosphatases. The difference in log(V_{max}) may be related to the origin of two types of phosphatase. Acid phosphatase is closely tied to root growth activity and plant demand for P (Tarafdar and Claassen, 1988), as plant roots are major producers of acid phosphatase (Speir and Cowling, 1991; Dinkelaker and Marschner, 1992; Krämer and Green, 2000). Microbes can produce and release large amounts of extracellular phosphatase due to their large biomass, high metabolic activity and short life cycles (Speir and Ross, 1978; Tarafdar and Claassen, 1988; Krämer and Green, 2000). Changes in alkaline phosphatase activity were related to changes in composition of bacteria communities (Renella et al., 2006). Alkaline phosphatase activity has not been detected in plants (Dick, 1983; Juma and Tabatabai, 1988). We found that enzymes originating from plants, e.g., acid phosphatase, had the highest enzymatic activity (Table 2), and the log(V_{max}) was significantly higher than that from microbial origins, e.g., alkaline phosphatase. This result is consistent with some previous studies. For example, the average log(V_{max}) values of various phosphatases were much greater in plant materials than those in soils and animal manures (Dick and Tabatabai, 1984). Krämer and Green (2000) found the maximum enzyme activity in five soils was observed at pH 4–6.5, where acid phosphatase had the highest activity. Nannipieri et al. (2011) also reported the range of enzyme activity for acid and alkaline phosphatase and acid phosphatase seemed to have higher values than the alkaline phosphatase (Nannipieri et al., 2011). But in high pH soils, alkaline phosphatase activity generally exceeds acid phosphatase activity (Eivazi and Tabatabai, 1977) and has been observed to be up to 6 times higher than acid phosphatase activity (Krämer and Green, 2000).

The log(V_{max}) values also varied considerably among the various materials studied (Dick and Tabatabai, 1984; Xie et al., 2010), similar to what we found in this study. While plants had the highest log(V_{max}), microbes (bacteria and fungi) had higher log(V_{max}) than wetlands (2.88) and soils. The average log(V_{max}) in soils was 2.61 μmol *p*NP g^{-1} h^{-1}, in the lower range of acid phosphatase activity measured in different soils (0.2–25.6 μmol *p*NP g^{-1} h^{-1}) (Olander and Vitousek, 2000). Rejsek et al. (2012) synthesized the acid phosphatase and reported the enzymatic activity for agricultural plots and forests can be as high as 86 or 360 μmol *p*NP g^{-1} h^{-1}. We found that enzyme activity was much higher in sludges than in soils. Nannipieri and Gianfreda (1998) also found that soil enzymes usually have lower V_{max} and higher K_m constants, indicating a lower catalytic efficiency and a reduced substrate affinity, than the respective purified enzymes from different sources. Criquet et al. (2007) reported that sewage sludges applied to soils increase phosphatase activity, implying that adding sludges stimulated microbial growth. The log(K_m) of the sludges was also higher than other origins. Plants, soils, fungi, lakes and wetlands had lower log(K_m).

4.2. Comparison of kinetic parameters under different incubation conditions

The expression and conservation of enzyme activity may be affected by natural environmental factors (e.g., seasonal changes, different geographic locations or diverse thermal soil regimes) and by anthropogenic activities (e.g., agriculture and management practices or pollution events) (Gianfreda and Ruggiero, 2006). But assay conditions such as presence or absence of a buffer, pH, substrates and their concentration, temperature, etc., could markedly affect the measured activity (Burns, 1978; Alef and Nannipieri, 1995). Usually, a soil enzyme assay is based on the use of a buffered solution of a synthetic, artificial substrate at a concentration high enough (saturation concentration) to be assumed constant throughout the time course of the enzymatic reaction and assuming a zero-order kinetics. So incubation conditions may or may not influence measured enzymatic activity. We tested whether enzymes incubated under different incubation conditions showed significant differences in log(V_{max}) and log(K_m). We found that unless a lower room temperature (20 °C) was used, no difference in log(V_{max}) was found among temperatures of 25 °C, 30 °C or 37 °C (Table 1). Thus, a commonly used temperature of 37° C seems to be adequate. Using 20 °C also produced lowest log(K_m). However, enzymes incubated with short time (10 min or 20 min) had higher log(V_{max}) and relative lower log(K_m). The standard time of 60 min produced lower log(V_{max}) and higher log(K_m). Comparative studies of incubation time often reported a more or less linear relationship between time of incubation and amount of *p*NP released, indicating that the assay is not complicated by microbial growth or

Table 3
Optimum pH (pH_{opt}) and sensitivity of pH (pH_{sen}) in the pH response functions for phosphatases.

Enzyme	pH_{opt}	pH_{sen}	Ratio of pH_{sen} and pH_{opt}
Acid	5.2 ± 0.1 (42)[a]	1.8 ± 0.2 (42)	0.38 ± 0.05 (42)
Alkaline	9.5 ± 0.3 (10)	2.6 ± 0.6 (10)	0.28 ± 0.06 (10)
All	6.0 ± 0.3 (52)	2.0 ± 0.2 (52)	0.33 ± 0.03 (52)

[a] Mean ± standard error (sample size).

assimilation of enzymatic reaction products by soil microorganisms. But some studies also proposed longer incubation, e.g., a 2-h incubation time was reported as adequate for the phosphatases (Verchot and Borelli, 2005).

Using *p*NPP as substrate produced higher log(V_{max}) and lower log(K_m) than others. Lower maximum substrate concentration tended to produce higher log(V_{max}), and the lowest substrate concentration might also overestimate log(K_m) (Table 1). Some plant originated enzymes in the S_{max} 5–15 mM group might contribute to the lower log(K_m) and higher log(V_{max}). A buffered condition is usually chosen when the disappearance of substrate and/or the formation of reaction products may change the pH of the solution, since an optimal pH value is required during the assay. Acid and alkaline soil phosphatase activities prevail in acid and alkaline soils, respectively (Eivazi and Tabatabai, 1977; Dick, 1983). Indeed, we found that different buffers significantly influenced log(V_{max}), probably due to different types of phosphatase involved. Sodium or Tris–HCl was most used for acid phosphatase while MUB was mostly used for alkaline phosphatase. Adding NaOH as a stopper during the incubation and measurements produced higher log(V_{max}) and lower log(K_m). Different wavelengths for absorption measurements also influence both the log(V_{max}) and the log(K_m).

Different assay protocols have been used in kinetic analysis of soil enzymes including phosphatase (Dick and Tabatabai, 1984; Burns et al., 2013). As we demonstrated here, differences in incubation condition may have significant influence on kinetic parameters. Thus, standardization of assay protocol is quite important, especially when we compare different enzyme activities or among different treatments.

4.3. Sensitivities of enzymes to temperature and pH

Activation energies are parameters that mechanistically link enzyme kinetics and temperature responses through the Arrhenius function. In the M–M function, the temperature sensitivity of V_{max} is directly related to the activation energy for the enzyme reaction (Davidson and Janssens, 2006; Wallenstein et al., 2011). A mechanistic model that includes temperature sensitivity of V_{max} would be superior to non-mechanistic empirical relationships, such as Q_{10}. As a purely empirical parameter, Q_{10} values are temperature-dependent and cannot be reliably extrapolated beyond measured response ranges or applied to novel systems, however, Q_{10} is still widely used as temperature sensitivity measurement.

We have studied the statistical features of E_a and Q_{10} that describe the impact of temperature on enzyme activity. The E_a value of acid phosphatases was significantly higher than that of alkaline phosphatases (Table 2). The estimated Q_{10} values mostly fell into the range of 1.2–2.3 and 1.1–2.0 for acid and alkaline phosphatases in this study, respectively. These results indicated that the temperature-driven changes in enzyme activity were greater for acid phosphatases than for alkaline phosphatases. Accumulated evidence of numerous studies suggests a wide range in temperature sensitivities for different enzymes, and measured Q_{10} values are often <2 (McClaugherty and Linkins, 1990; Frankenberger and Tabatabai, 1991; Wirth and Wolf, 1992; Criquet et al., 1999; Trasar-Cepeda et al., 2007). These findings are consistent with our results. While being used to model the dependence of mineralization rate on temperature, E_a is usually regarded as invariant. The assumption that all enzymes are equally sensitive to temperature has not been borne out in the literature (Wallenstein et al., 2011). Our results indicated that E_a might vary for different phosphatases or under different environmental conditions (Wallenstein et al., 2011). The mean E_a was found to be 34.40 kJ mol^{-1} (corresponding to an E_a of 0.36 eV and $Q_{10} = 1.72$ with base $T = 20$ °C), similar to the mean E_a for soil extracellular enzymes that are not conformationally constrained (Wallenstein et al., 2011).

The means of both pH_{opt} and pH_{sen} were significantly lower for acid phosphatases than those for alkaline phosphatases. When the exponential-quadratic function (Eq. (6)) was used to describe the pH impact on enzyme activity, the average ratio of pH_{sen} to pH_{opt} was 0.38 ± 0.05 for acid phosphatases. This means that a pH increase or decrease of 1.6 pH units ($\approx 0.3 \times pH_{opt}$) from pH_{opt} would reduce the enzyme activity by 50%, and an increase or decrease of 3.0 ($\approx 0.6 \times pH_{opt}$) would result in a decrease of 90% in enzyme activity (Wang et al., 2012).

4.4. Limitations and future research

We focused on temperature and pH effects on the enzyme activity in this study. Temperature and pH may also influence the half-saturation constant (K_m) (Davidson et al. 2006). However, due to the limitation of data availability, we could not derive their relationships here. Many other factors such as microbial biomass, soil organic carbon and phosphorus concentrations, as well as the C:N:P ratios of the organic matter may also influence enzyme activities (Waldrop et al., 2000; Henry, 2012; Hobbie and Hobbie, 2012; Sinsabaugh et al., 2002; Burns et al., 2013). The relationships between enzyme activities and these factors are still not quite clear. As recently pointed out by Burns et al. (2013), the effects of soil biotic and abiotic environment on enzyme activities and their contribution to global biogeochemical processes need to be further studied.

We are still not clear on the relationships between the model parameters estimated in incubations with artificial substrates and the enzymes activities in natural ecosystems. Very few ecosystem models have considered the role of phosphatase in phosphorus (P) mineralization because of a lack of understanding of phosphatase-mediated process and phosphatase kinetics. Wang et al. (2007) developed a biogeochemical model that describes the biochemical P mineralization using the Michaelis–Menten kinetics. The model considers that both the production of phosphatase by plant roots and their function in the biochemical P mineralization rate are dependent on the nitrogen availability (Almeida et al., 1999). Soil N:P ratio may influence P mineralization. In some cases, N:P ratios are also not constant among different biomes and have different effects on enzyme activity (Xu et al., 2012; Yang and Post, 2011). Additionally, to understand the true enzyme activities in natural ecosystems, new *in situ* methods need to be developed. Burns et al. (2013) considered this is one of the important issues need to be studied in the future. However, we may expect that the parameters estimated in incubations should be closely related to and can be upscaled to the enzymatic activities given a certain enzyme type and environmental condition. If this kind of scaling method and the interdependence between nitrogen and phosphorus dynamics could be developed, we can use the two kinetic parameters compiled in this study to parameterize the soil nutrient cycling models.

5. Conclusions

We compiled a kinetic parameter database for phosphatases from literature. The variance analysis and normality test indicated that both V_{max} and K_m were log-normal distributed. Significant differences in V_{max} were found between acid phosphatase and alkaline phosphatase and among different origins (e.g., plant and soil). Significant difference in activation energy existed between acid and alkaline phosphatase (36.30 and 23.61 kJ mol^{-1}, respectively). The exponential–quadratic function can be used to describe the effect of pH on the enzyme activity. Acid and alkaline

phosphatases had quite different pH_{opt} and pH_{sen}. The average ratio of pH_{sen} to pH_{opt} was 0.38 for acid phosphatase and 0.28 for alkaline phosphatase. The information provided a general picture of phosphatase and could be useful for modeling of phosphorus cycling. The results also indicated that standardization of assay protocol of phosphatase is necessary to provide better estimation of kinetic parameters.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.soilbio.2013.05.017.

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