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Letter to the Editor

# Response to Steen and Ziervogel's comment on "Optimization of hydrolytic and oxidative enzyme methods to ecosystem studies" [Soil Biology & Biochemistry 43: 1387–1397]

Steen and Ziervogel provide a timely and useful comment to augment what we have presented in our original review article. We completely agree with their points and data, and in fact, we have observed similar trends with alkaline phosphatase activity in sea water samples collected from the Pacific Ocean off Southern California (Fig. 1). Interestingly, the inhibitory effects of the substrate depend on assay temperature. We have also observed substrate inhibition in assays of cellobiohydrolase and leucine aminopeptidase, two other hydrolytic enzymes commonly measured in environmental samples. However, substrate inhibition was only observable by measuring enzyme activity across a range of substrate concentrations, a step that is often neglected in enzyme assays with environmental samples. We decide on the best substrate concentration to use for analysis only after performing preliminary assays and confirming the concentrations at which activity is maximal, similar to what Steen and Ziervogel suggest for $[S]_{opt}$ and $V_{opt}$. For instance, in a recent study of Michaelis–Menten kinetics of enzymes in different soils, the range of substrate concentrations used varied by enzyme (and in some cases, location) due to substrate inhibition in some enzymes (German et al., in press).

In our review article we state that "it is important to confirm that each hydrolytic enzyme is assayed under saturating conditions, as activities measured at lower substrate concentrations will underestimate potential digestive enzyme (DE) activity" (German et al., 2011). Although avoidance of activity underestimation is the primary driving force behind performing Michaelis–Menten curves in this example, avoiding substrate inhibition is also inherent in this analysis; one cannot generate the hyperbolic curve necessary for Michaelis–Menten kinetics if the activity levels decrease at higher substrate concentrations (Fig. 1). Thus, by constructing substrate saturation curves, one ensures $V_{opt}$ is being measured (by avoiding substrate concentrations that become inhibitory). Steen and Ziervogel's comment strengthens the case for researchers to ensure they confirm the appropriateness of chosen substrate concentrations before engaging in a specific study of enzyme activities in environmental samples. Moreover, their argument regarding cooperative binding and measuring activities at low substrate concentrations further supports the need for measuring DE activity across substrate concentration gradients.

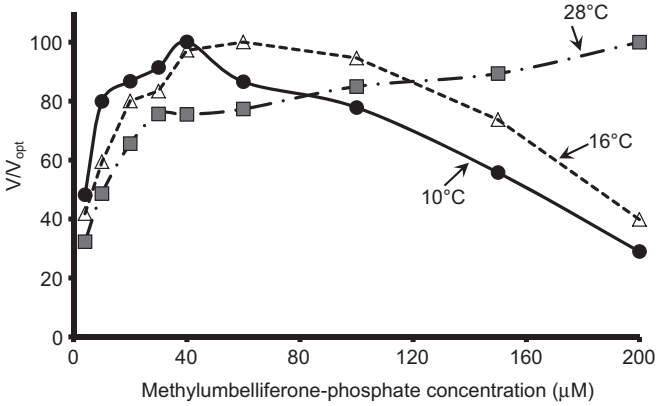


**Fig. 1.** Alkaline phosphatase activity in Pacific Ocean water as a function of substrate concentration. Water was sampled from Newport Beach, CA (33° 36′ 21.77″N, 117° 55′ 51.96″W) on 30 April 2010. Activity was measured at three different temperatures and is normalized (as a percentage) to Vopt for each sample. The lines serve as visual guides and are not the result of non-linear regression.

With regard to the oxidase assays, we agree that much is left to be determined for these enzymes (Sinsabaugh, 2010). We did make the statement that "swamping the sample with an overabundance of substrate will allow the enzymatic reaction to proceed at some maximal rate, whatever that might be." However, we agree that substrate inhibition could also occur with these enzymes, and substrate concentration curves are warranted for oxidative enzymes as well as for hydrolytic enzymes.

Although not discussed at length in our review article, we recognize that the Michaelis–Menten constant ($K_m$) measured in environmental samples is an "apparent $K_m$" because it represents the $K_m$ of a conglomeration of hydrolytic enzymes acting on a specific substrate in an *in vitro* enzyme assay (Sinsabaugh and Follstad Shah, 2010). There are a suite of enzymes in soils (and other environmental samples) that are either secreted by living microorganisms or stabilized in the soil-organic matter matrix. Moreover, there are clear kinetic differences between recently secreted and stabilized enzymes in soils (e.g., Marx et al., 2005). In our review article we do acknowledge that there is value in studying enzymes in different fractions of soils (page 1388, at the end of the Introduction section), but we chose to defer to previous reviews on this topic to keep the article focused (German et al., 2011). Furthermore, the apparent $K_m$ observed in bulk samples is appropriate to measure because it provides an estimate of the potential inherent in a system (e.g., see Sinsabaugh and Follstad Shah, 2010; Stone et al., in press; German et al., 2011). No matter the outcome, measuring enzyme activity across a gradient of substrate concentrations provides insight into ecosystem function that is unavailable if substrate concentration is chosen based solely on published methods. Observations of substrate inhibition, cooperative binding, or non-Michaelis–Menten kinetics can only be made by varying substrate concentrations in enzyme assays, and we agree that more researchers should perform such analyses with their samples.

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