Excessive accumulation of phosphorus (P) in manure-amended agricultural soils can lead to downstream eutrophication and the subsequent destruction of water quality. The forms of phosphorus found in the soil effects the long term potential for the soil to leach damaging amounts of P. Rapid and accurate measurement of these forms of soil P precludes any remediative efforts. Enzymatic hydrolysis (EH) is an emerging method for accurately characterizing P forms in many different types of environmental samples, including manure-amended soils. EH is only trumped in accuracy by ³¹P-NMR spectroscopy—a method that is both expensive and fails to measure the long term potential of a soil to release phosphorus. We have adapted an enzymatic hydrolysis method capable of measuring four classes of phosphorus (simple monoester P, phytate P, nucleic acid P and inorganic P) to a micro-plate reader system and verified the effectiveness of this adaption by comparing EH measures to ³¹P-NMR measures of identical samples. Enzyme cocktails were freeze-dried into standard 96-well plates and used to accurately and efficiently measure P forms in many samples at one time. The utility of this method has been demonstrated by measuring the P composition of an unprecedented 210 agricultural soil samples from the Mississquoi Bay watershed that have been subjected to long-term dairy manure amendment. Manure management strategies will be enhanced by watershed-scale P distribution models that could be generated using this technology.