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Application of *para*-nitrophenol (*p*NP) enzyme assays in degraded tropical soils

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Abstract

Enzyme activities have been used as indicators of soil quality and changes in biogeochemical function due to management or perturbations. The objective of this study was to answer a number of methodological questions regarding sampling schemes, sample handling recommendations, and assay procedures to facilitate the use of enzyme assays in the tropical highlands of East Africa. We used *para*-nitrophenol (*p*NP) based substrates for five enzymes: β-glucosidase, cellobiohydrolase, chitinase, acid phosphatase, and alkaline phosphatase. In the first experiment, we examined sampling procedures and compared the results of determining enzyme activities on a plot using composite or discrete samples. Composite samples usually had higher activities than the means of individual cores ($P<0.05$), but relative ranking of sites was the same if analyses were based on composite or discrete samples. In the second experiment, we examined the effects of storage time and conditions on enzyme activity. Enzyme activity degraded rapidly in frozen samples, but was better maintained in samples stored at 4 °C. Phosphatase and cellobiohydrolase activity declined after 14 days of storage, while the activity of the other enzymes remained close to the values of fresh samples for 28 or more days. In the third experiment, we examined the effect of the addition of an antiseptic, toluene, to prevent bacterial growth during the assay. We found no consistent toluene effect ($P>0.4$), probably because the assays were of short duration and microbial growth was minimized. Finally, we looked at the incubation time necessary to produce reliable results. Phosphatases, with relatively high activities could reliably be determined in 2 h incubations, but the other enzymes had much lower activities and required longer incubation times for reliable determination. For the enzymes we looked at, 4 h was a good standard time for determining the activity of even the lowest activity enzymes. The results of this study provide practical guidelines for applying these enzyme assays in the degraded tropical soils.

Keywords: Microbial function; Soil enzymes; Extracellular enzymes

1. Introduction

Microbes mediate important biochemical transformations associated with nutrient cycling in soils. Since enzymes catalyze all biochemical transformations, measurements of soil enzyme activities are useful indicators of biological activity. Soil enzyme activity measurements have been used as indices of land quality and soil health (Dick, 1984; Kennedy and Papendick, 1995; Bergstrom and Monreal, 1998; Bandick and Dick, 1999; Badiane et al., 2001; De la Paz Jimenez et al., 2002), as well as to understand how human activity is changing biogeochemical cycles in ecosystems (Bolton et al., 1985; Bergstrom et al., 1998; Carreiro et al., 2000; Wick et al., 2000; Saviozzi et al., 2001). There are some controversies about the interpretation of enzyme assays, particularly regarding how well current methods separate extracellular activities from intracellular activities (Nannapieri et al., 2002). In addition, laboratory activity determinations often optimize conditions for soil enzymes and it is not clear how these activities relate to biological function in situ.

Application of soil enzyme activity assays has been widespread in the relatively fertile soils of North America (Tabatabai, 1994; Bergstrom et al., 1995; Dick et al., 1996a,b) and Western Europe (Garcia et al., 1994), where commercial fertilizers have been used to supply essential nutrients for adequate crop production. Few studies have used these techniques in degraded tropical soils. There are a number of sampling and assay method questions that need to be answered before these methods are applied in these systems.

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At a very simple level, an enzyme assay consists of adding a known amount of soil to a solution containing a standard concentration of a substrate and measuring the rate at which the substrate is converted to a product. Conditions of the assay must be controlled with respect to temperature, pH, and ionic strength of the solution (Tabatabai, 1994). There are a number of published assay protocols, most of which vary slightly to optimize the procedure for the soil that is being studied. This study aims to validate existing sampling methods and enzyme assay procedures from temperate ecosystems, to provide specific recommendations for application of these measurements to tropical agricultural soils. The investigation focuses, in particular, on four methodological aspects, which have been reported to have a significant effect on the determination of enzyme activities. The first two involve sample collection and handling before analysis: (i) spatial variability at the plot level, and the implications of this for sampling, and (ii) the effects of storage time and conditions on enzyme activity. The second two pertain directly to how the assay is performed: (iii) the effect of the addition of an antiseptic to prevent bacterial growth during the assay, and (iv) the effect of incubation time on enzyme activity.

Five soil enzymes representative of important nutrient cycles were chosen for this study. Both β-glucosidase and cellobiohydrolase play important roles in C cycling (Eivazi and Tabatabai, 1990). The first hydrolyses carbohydrates with β-D-glucoside bonds, such as maltose and cellobiose (Klose and Tabatabai, 2002), producing sugars, an important energy source for soil microorganisms (Eivazi and Tabatabai, 1990). Cellobiohydrolase is a cellulolytic enzyme that breaks down cellulose through the removal of glucan units from the end of the cellulose chains. β-D-acetyl-glucosaminidase, or chitinase, is the second enzyme in a chain of three required in the degradation of chitin, a major reservoir of organic C and N in soils. By hydrolyzing chitobiose, it releases low molecular mass C- and N-rich compounds into the soil (Sinsabaugh and Moorhead, 1994; Ekenler and Tabatabai, 2003). Phosphatases play a key role in phosphorous mineralization, and P cycling. They are ubiquitous in soil and have been studied extensively because they catalyze the hydrolysis of organic phosphomonoester to inorganic phosphorous, making it available for plant uptake (Tabatabai, 1994; Alef and Nannipieri, 1995; Amador et al., 1997). According to their optimum pH, phosphatases are classified as acid (orthophosphoric monoester phosphohydrolase, pH 6.5) or alkaline (orthophosphoric monoester phosphohydrolase, pH 11).

2. Materials and methods

2.1. Site description

Soil samples were collected in early May 2002 from two field experiments in Western Kenya, representing contrasting soil types. The mean annual temperature for both sites is 25 °C. The first site, Ochinga Farm, is situated at 0°06′N and 34°34′E at an elevation of 1420 m ASL. Rainfall is bimodal with an annual mean of 1800 mm. Soil is a highly weathered Nito-Humic Ferralsol that is slightly acidic, with a pH_{water} of 5.1 (2.5:1 water to soil) and is composed of 26% sand, 28% silt, and 46% clay (Maroko et al., 1998). Organic C contents in the soils sampled ranged from 1.4 to 1.6%. The second site, Teso Farm, lies at an altitude of 1290 m ASL and is situated at 0°35′N and 34°12′E. The mean annual rainfall recorded at the site is 1200 mm. The soil is classified as a Ferralo-Orthic Acrisol and is composed of 71% sand, 12% silt, and 17% clay, with a pH_{water} of 5.4. Organic C contents in the soils sampled ranged from 0.9 to 1.1%.

The sites are part of a larger experiment to evaluate improved fallow practices on agricultural productivity and soil erosion (Boye, 2000). Experimental design is a randomized complete block, with treatments consisting short rotation fallows with different tree species. For these measurements, we chose two of the treatments from these experiments: improved fallow systems under *Tephrosia candida* and continuous maize cultivation. The continuous maize treatment in this experiment is an absolute control, with no soil amendments, and represents degraded agriculture; the improved fallow site represents rehabilitated agriculture. The fallow treatments follow a rotation between maize and tree-legume fallow. At the beginning of the experiments, improved fallows were planted for 18 months to rehabilitate the sites. The subsequent rotation consisted of direct sowing of the *T. candida* under the maize crop in May, during the long rainy season, at the 8-leaf stage of the maize crop. The *T. candida* is left in the field following the maize harvest in July, and is allowed to grow through the short rainy season (September to November). The fallow is then cut prior to cultivation, in February of the following year, and maize is sown in March. Leaf biomass of the fallow is incorporated in the soil at the time of cutting.

2.2. Sampling procedure

Samples were collected at regular intervals along a grid in each plot, to a depth of 5 cm. At Ochinga, on plots measuring 3.0×12.0 m, samples were collected at 1.0 m intervals along the plot's width and 2.0 m intervals along its length. In Teso, where plots were slightly larger, measuring 9.0×16.0 m, samples were collected at a distance of 2.5 m along the plot's width and at 3 m intervals lengthwise. Within each plot, individual samples were mixed to form one composite sample per plot for all experiments except the spatial variability experiment.

2.3. Enzyme assays

The assay methods used were modified from Tabatabai and Bremner (1969) according to Sinsabaugh and Linkins (1990);

Table 1
Substrates used for each enzyme assay

Enzyme assayed	Substrate
β-glucosidase	*p*NP-β-D-glucopyranoside
Chitinase	*p*NP N-acetyl-β-D-glucosaminide
Cellobiohydrolase	*p*NP-β-D-cellobioside
Acid phosphatase	*p*NP-phosphate (buffer pH 5.0)
Alkaline phosphatase	*p*NP-phosphate (buffer pH 9.0)

Carreiro et al. (2000). These methods use *p*NP linked substrates and enzyme activity is determined from colorimetric measurements of *p*NP released when soil is incubated in a buffered substrate solution. The substrates used for each enzyme assay are listed in Table 1.

For each sample, 15 g of soil per sample was mixed with 100 ml of 0.05 M acetate buffer (pH 9.5 for alkaline phosphate; pH 5.0 for all other enzymes) in 250-ml Nalgene bottles. For all experiments in this paper, three lab replications were run, each beginning with a separate slurry. Two millilitres aliquots of slurry were pipetted from each slurry into polypropylene test tubes, which were kept chilled pending incubation. At the beginning of each incubation, 2 ml of substrate (5 mmol for all substrates except *p*NP-β-D-cellobioside which was prepared at 2 mmol concentration) solution were added to each sample test tube. The tubes were then capped and placed on a rotary shaker to be mixed for two hours during incubation at 25 °C. Following incubation, tubes were centrifuged for 5 min, and a 1 ml aliquot of clear supernatant was transferred from each tube to a 15 ml glass test tube containing 0.2 ml of 1 N NaOH, to stop the reaction and cause color change. The solution was brought to a final volume of 10 ml using deionized water, vortexed and light absorbance measured with a spectrophotometer at 410 nm. Sample and substrate controls were run during each incubation to control for color development due to the substrate or dissolved humic substances. Controls were made by mixing 2 ml of acetate buffer with either 2 ml of soil slurry or 2 ml of substrate solution. The concentration of *p*NP detected in samples after incubation was corrected by subtracting the combined absorption results for the sample and substrate controls from the analytical samples. Gravimetric water content was determined by drying soils at 105 °C for 24 h. All results were reported in units of μmol *p*NP g-soil^{-1} h^{-1}.

2.4. Experiment 1: effect of spatial variability

To assess whether composite sampling yields information that is truly representative of the sampling area, we compared the mean of individual samples collected on the grid for each plot with the value obtained from the composite sample. Spatial variability was further quantified using the coefficient of variation. The means of the individual cores were compared with the value obtained using a composite sample by means of a *t* test.

2.5. Experiment 2: effect of storage

After soil samples were returned to the laboratory, they were thoroughly mixed and separated into two sub-samples. Sub-samples were stored in plastic bags, either under cold (4 °C) and frozen conditions (−20 °C) to be subsequently analyzed at days 3, 6, 14, 28, 42 and 56. Results were then compared to values obtained from immediate analysis of field-moist soils on the same day of collection (day 0). Analysis of a storage effect was done with an ANOVA.

2.6. Experiment 3: effect of toluene

For this series of experiments, 3 ml of the microbial growth inhibitor, toluene, were added to the soil slurries before beginning the incubations. A second set of slurries was prepared without toluene. Comparisons were made using a paired *t*-test to determine whether the toluene treatment had a significant effect on the assay results. Results were analyzed graphically.

2.7. Experiment 4: effect of incubation time

Published procedures recommend that buffered substrate solutions be incubated for 2 h at a known temperature (Tabatabai and Bremner, 1969; Carreiro et al., 2000). For this series of experiments we varied the incubation time between 1 and 12 h. Twelve tubes were prepared from each slurry and one was selected at random each hour for analysis.

3. Results

3.1. Experiment 1: effect of spatial variability

Coefficients of variation for the different enzyme assays ranged between 20 and 60% (Table 2). Maize plots at Ochinga (fine texture) showed generally higher coefficients of variation than the improved fallow plots. At Teso (coarse texture), the reverse was true; coefficients of variation were generally higher for the fallow plots. Variability differed between enzymes, such that two groups can be distinguished: a higher variability group including β-glucosidase, chitinase and cellobiohydrolase, and a lower variability group comprising acid and alkaline phosphatase. Variability in the latter two enzymes was relatively constant across sites and across treatments.

To test whether compositing samples can yield a reliable estimate of biochemical measurements at the plot level, we compared the mean of the composite sample with the mean of individual soil cores taken at regular intervals along the plot. Generally, enzyme activities in the composite samples were significantly greater than in the individual samples (Table 3).

Table 2
Means (μmol *p*NP g-soil^{-1} h^{-1}) and coefficients of variation (%) for enzyme activity values of the spatial variability samples in Ochinga and Teso farms

Parameter	Fallow				Maize			
	Ochinga		Teso		Ochinga		Teso	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
β-glucosidase	0.29	30.4	0.09	57.6	0.07	40.2	0.18	58.4
Chitinase	0.23	30.1	0.05	46.0	0.07	42.1	0.13	22.1
Cellobiohydrolase	0.06	24.1	0.03	49.9	0.01	50.2	0.04	46.2
Acid phosphatase	1.27	21.1	0.64	30.7	0.58	23.2	1.04	22.1
Alkaline phosphatase	0.96	31.4	0.70	28.1	0.48	23.3	1.12	20.6

3.2. Experiment 2: effect of storage

Enzymes differ in their level of susceptibility to storage conditions and this sensitivity varies with management and soil type. We analyzed this experiment using an ANOVA that controlled for sources of variation associated with site, block and treatment. In samples stored at 4 °C, for a number of enzymes, there was an initial drop in activity at day 6, followed by a peak after 2 weeks and a constant decline in activity over the subsequent 8 weeks (Fig. 1). β-glucosidase, cellobiohydrolase and chitinase form a distinct group, with much lower activity. β-glucosidase activity declined slowly over the first 42 days of storage at both sites, but was not significantly different from the initial activities ($P>0.05$). On the clay soil at Ochinga, the activity dropped off significantly in the final measurement. The other enzymes showed no significant ($P>0.05$) decrease in activity over the first 14 days of storage, with the exception of the day 6 measurement.

For all enzymes, frozen storage, even for very short periods, greatly reduced enzyme activity (Fig. 2). Activity remained relatively constant during the storage period after the initial decline. In all cases, except for cellobiohydrolase, assay results obtained on day 0 were significantly higher ($P<0.05$) than the results obtained on all other days. Additionally, the relative site ranking was less consistent for the samples stored frozen than it was for the samples stored at 4 °C.

3.3. Experiment 3: effect of toluene

The effect of the addition of the microbial growth inhibitor, toluene, was tested for each enzyme using an analysis of variance that controlled for variation associated with the crop, site and block. Toluene had no effect on any of the enzyme activities assayed (Table 4). However, there was a general tendency towards elevated values in the incubations conducted without toluene. A multivariate analysis of variance (MANOVA) showed no significant toluene effect for the ensemble of the enzymes assayed (Wilk's lambda = 0.823, $P=0.698$).

3.4. Experiment 4: effect of incubation time

Most enzyme activities were relatively constant with varying incubation times, but a few of the enzymes, cellobiohydrolase in particular, showed increasing enzyme activity with time (Fig. 3). During short incubation times, most enzymes released such low quantities of product that concentrations were below the lowest standard and measurements of enzyme activity were therefore unreliable. The amount of *p*NP released was particularly low for

Table 3
Means of enzyme activities (μmol *p*NP g-soil^{-1} h^{-1}) determined on individual soil cores and composite values, with associated probabilities for the comparison between the two sampling regimes for Ochinga and Teso farm plots

Parameter	Fallow			Maize		
	Core mean	Composite value	*P*	Core mean	Composite value	*P*
Ochinga						
β-glucosidase	0.29	0.45	<0.001	0.07	0.12	<0.001
Chitinase	0.23	0.29	0.0021	0.07	0.10	0.0010
Cellobiohydrolase	0.06	0.07	0.0003	0.01	0.01	0.0302
Alkaline phosphatase	0.96	2.03	<0.001	0.48	0.92	<0.001
Acid phosphatase	1.27	1.96	<0.001	0.58	0.81	<0.001
Teso						
β-glucosidase	0.09	0.19	<0.001	0.17	0.26	0.0028
Chitinase	0.06	0.18	<0.001	0.13	0.19	<0.001
Cellobiohydrolase	0.02	0.03	0.1075	0.04	0.04	0.4891
Alk phosphatase	0.70	1.13	<0.001	1.12	1.50	<0.001
Acid phosphatase	0.64	0.87	0.0002	1.04	1.18	0.0167

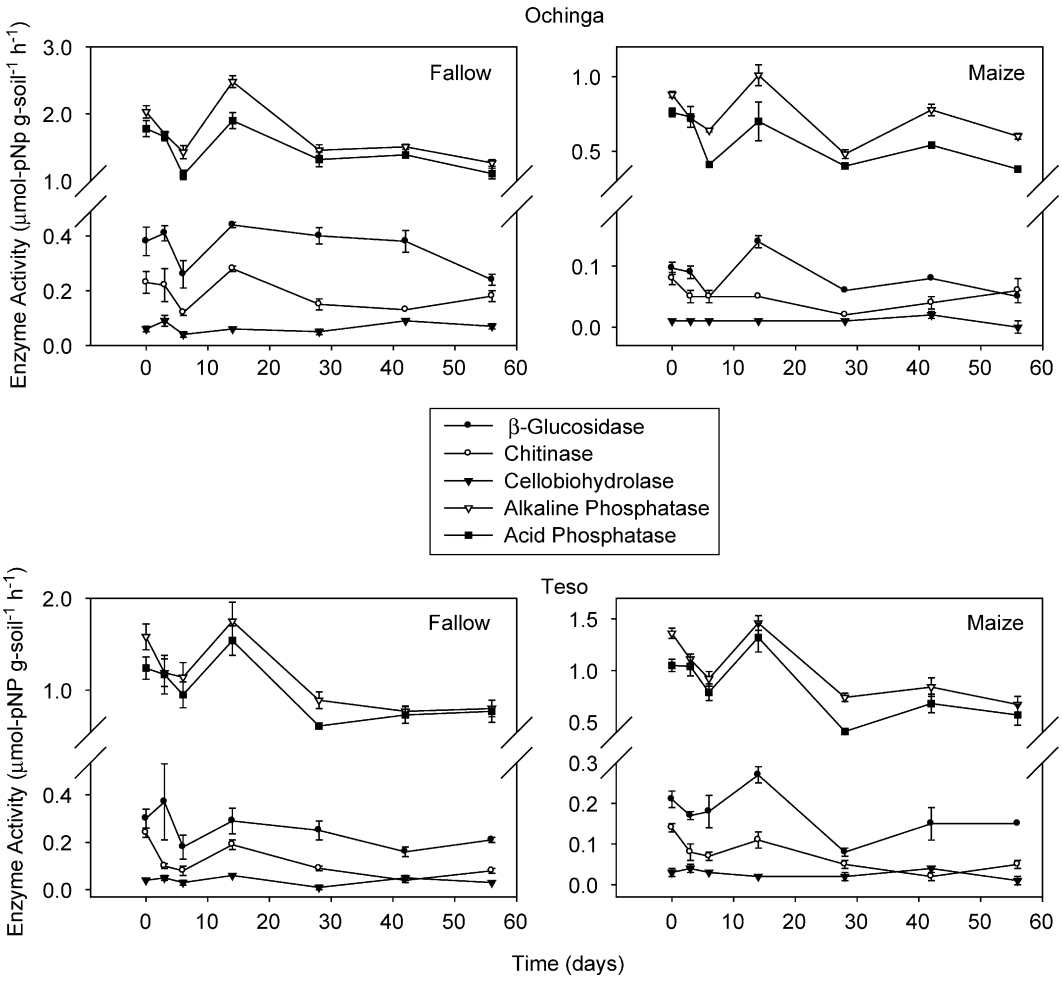

Fig. 1. Changes in enzyme activity of under cold storage conditions (4 °C) over a period of 8 weeks.

the cellobiohydrolase enzyme, and even after 12 h of incubation, concentrations of *p*NP were only one-tenth of the concentration of the lowest standard. Incubation times of more than 10 h generally resulted in readings that were off the scale for acid and alkaline phosphatase, so that samples had to be diluted at a later stage to enable accurate measurements. Plots cultivated with *T. candida* in Ochinga farm recorded the highest levels of enzyme activity for all enzymes.

4. Discussion

Because of the inherent complexity of soils, with properties that vary both spatially and temporally, sampling procedures designed to investigate indicators of soil biological activity, such as enzymes, must be carefully tested. The aim of this study was to provide a standardized set of methods, which could be used in tropical agricultural soils to determine soil quality thresholds and provide information on the variability and ranges of values that can be expected when performing enzyme assays. As far as values are concerned, observed ranges were consistent with data published by Dick et al. (1996) for all enzymes.

Enzymes are highly variable over short distances (Webster and Oliver, 1990), and their activity is linked to the distribution of other soil properties such as moisture and organic matter content (Jordan et al., 1995; Bergstrom et al., 1998). Enzyme activity is also influenced by management practices (Bolton et al., 1985; Dick, 1992; Ajwa et al., 1999; Bandick and Dick, 1999; Ekenler and Tabatabai, 2003), drainage, and the distribution of root biomass (Amador et al., 1997). In the current investigation, enzyme activities differed in their variability. Phosphatases showed the lowest levels of spatial variability, with CVs ranging from 20.6 to 30.7%. These values are consistent with observations recorded by Bonmati et al. (1991) in grasslands and Amador et al. (1997) in a riparian forest system. The other three enzymes had higher coefficients of variation, ranging between 20 and 60%, but were generally greater than 45%. Decker et al. (1999) showed that β-glucosidase, acid phosphatase and chitinase activities were highly variable at the plot level and that this variation was linked with variation in organic matter content. Bergstrom et al. (1998)

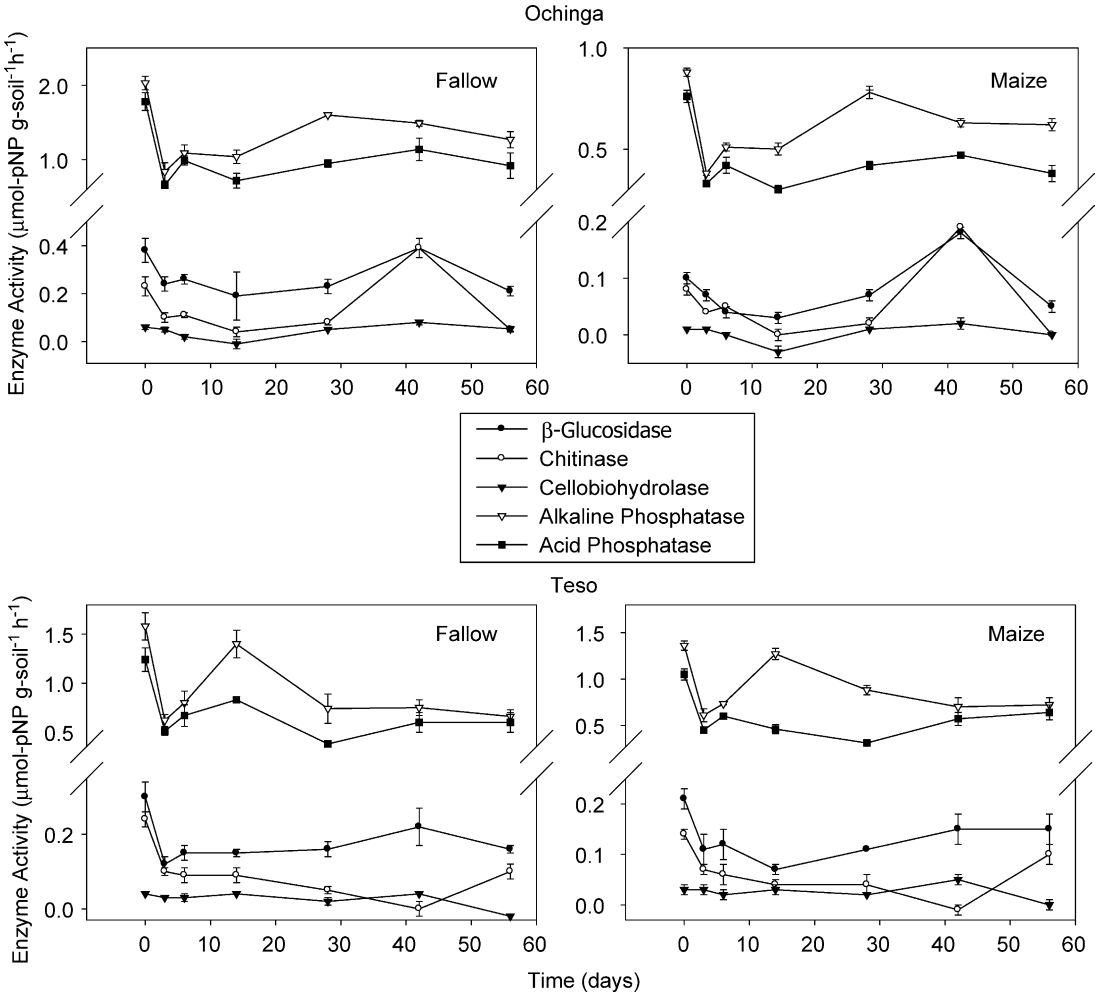

Fig. 2. Changes in enzyme activity under frozen storage conditions (−20 °C) over a period of 8 weeks.

found similar variability on a conventionally tilled field, when estimating β-glucosidase, urease, phosphatase, dehydrogenase, and arylsulfatase activity. In the current study, highest levels of variation in β-glucosidase were exhibited on the sandy soil, for both the maize and the improved fallow treatments. Results from this study seem to confirm previous findings that indicate the importance of soil type and, to a lesser extent, land-management practices on variability of enzyme activities. They also indicate that sampling procedures need to be designed to account for higher spatial variability on sandy soils.

When evaluating the effectiveness of composite sampling as a reliable collection method for tropical soils a number of considerations have to be made. Because soil sampling is costly and time-consuming, composite sampling considerably reduces the number of soil samples to an acceptable level of sampling and analytical costs. Unless there is strong evidence for non-uniformity within the sampling area (e.g. obvious changes in soil type, topography and land use), composite sampling is considered one of the most effective sampling methods. Due to its composite nature, however, one cannot estimate the variance of the mean and consequently the error associated with an estimate within a plot. As a rule of thumb, combining a smaller number of samples provides less reliable estimates of a population's parameters than combining larger amounts. Studies have shown, nevertheless, that small uniform areas, measuring less than 0.5 ha can be sampled with as few as 5–10 samples and that larger areas gain little in precision when numbers are greater than 25 (Webster and Oliver, 1990). In two studies on spatial variability of various enzymes Bonmati et al. (1991) and Speir et al. (1984) found that sample variability was

Table 4
Means (SE) of enzyme assays with and without toluene addition, and associated probabilities for the comparison between the two procedures

Parameter	*N*	No toluene	Toluene	*P*
β-glucosidase	24	0.25 (0.04)	0.21 (0.03)	0.448
Chitinase	24	0.17 (0.02)	0.14 (0.01)	0.699
Cellobiohydrolase	24	0.04 (0.01)	0.03 (0.00)	0.528
Alkaline phosphatase	24	1.46 (0.14)	1.33 (0.10)	0.442
Acid phosphatase	24	1.21 (0.12)	1.05 (0.08)	0.348

Mean enzyme activity is expressed as µmol of *p*NP released g-soil^{-1} h^{-1}.

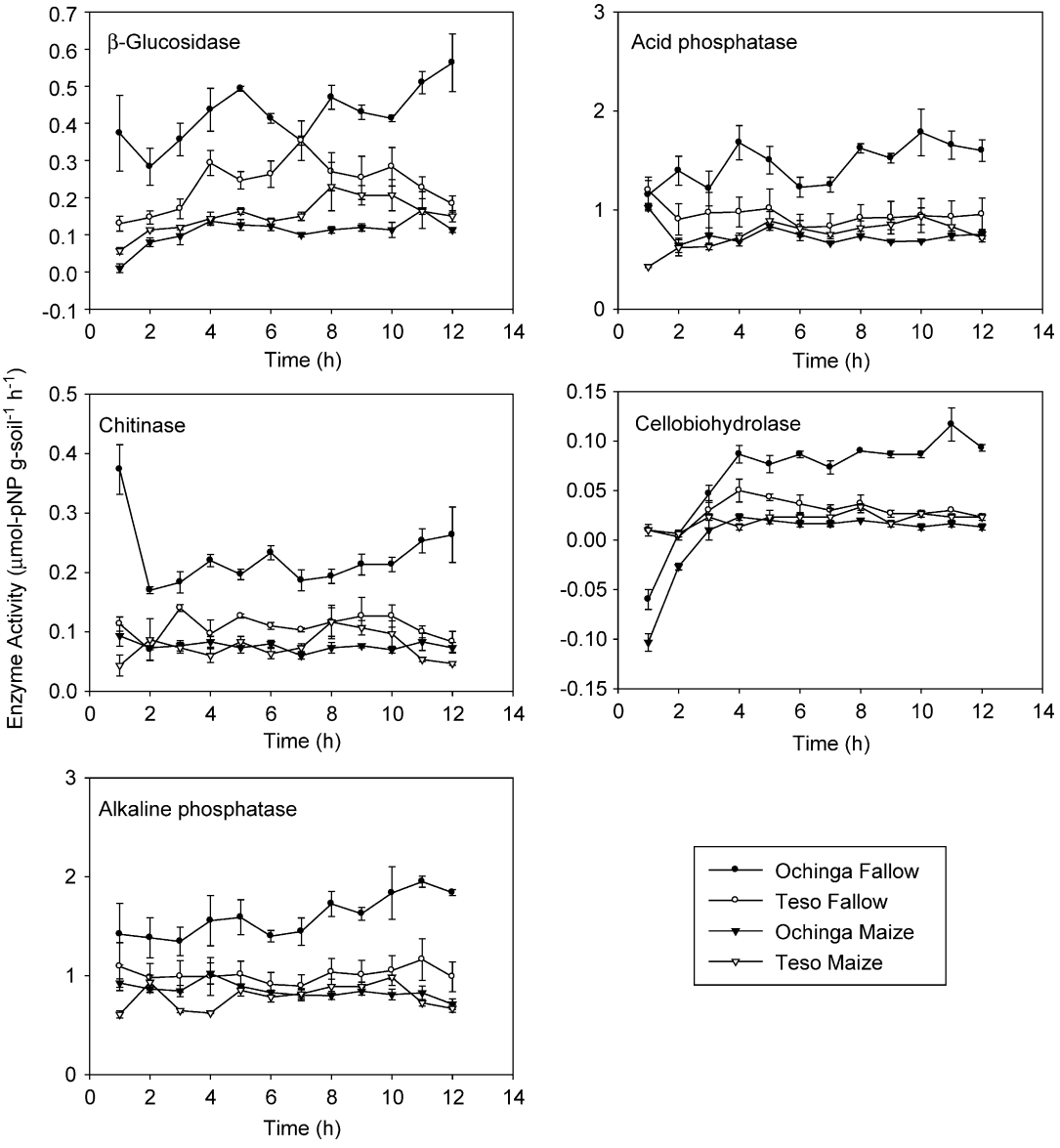

Fig. 3. Measured enzyme activity of as a function of incubation time.

generally higher than assay variability. In the second study, the mean of individual samples was not significantly different from a composite sample. In this study, activities measured in composite samples were higher than the averages calculated from individual soil cores, but the general trends observed in the individual samples were mostly preserved in the composite data. An 18-core sample, such as the composite sample taken in this study adequately estimates the mean site value for all enzyme properties with standard errors of between 5 and 15%. We conclude that, when the objectives are to obtain a relative index of enzyme activities between plots or treatments, composite samples can be used. However, for absolute comparisons between sites or soil types, individual soil cores must be analyzed.

Storage of soils is often necessary for practical reasons following collection. The concern is that storage will result in a decline in enzyme activity as extracellular enzymes degrade and microbial community composition changes. In this study, storage under cold and frozen conditions confirmed results from previous studies, which found that susceptibility to storage is highly dependent on the property being measured (Speir and Ross, 1975; Stenberg et al., 1998; Verchot, 1999). Phosphatases appeared to be the most vulnerable of enzymes tested, with activity values showing a decline after 2 weeks. If possible, therefore, one should test for phosphatase activity as soon after soil collection as possible. β-glucosidase, cellobiohydrolase and chitinase showed much lower levels of activity reduction due to storage, although for all enzymes storage at 4 °C was markedly preferable to storage at −20 °C. Microbial communities in Western Kenya never experience temperatures below zero, under normal climatic conditions, thus

making them particularly susceptible to freezing. We can conclude that although storage produced changes in absolute activity measures for each enzyme, relative values were not affected and differences within and between sites were maintained throughout the study. It is recommended, therefore, that if samples from agricultural tropical soils cannot be tested on the same day of collection, they be stored at 4 °C until suitable time for analysis. Relative differences between and within sites will still be detected, providing analysis for individual enzymes are performed on the same day or within a few days of each other.

Toluene has been used in enzyme assays because of its effectiveness in reducing residual microbial growth and inhibiting further enzyme synthesis during incubation. However, toluene has a plasmolytic effect on microbial cell membranes and it has also been reported to promote the release of intracellular enzymes, and consequently cause an increase in measured enzyme activity. Furthermore, its effect varies with soil, experimental conditions and enzyme assayed (Frankenberg and Johanson, 1986; Tabatabai, 1994). Results from our study showed that toluene was indeed effective in preventing microbial growth, with treated samples exhibiting lower values than untreated ones. However, in all but alkaline phosphatase, differences between the two treatments were not statistically significant. This confirms data published by Frankenberger and Carreiro et al. (2000) Tabatabai (1994) who found that toluene had little or no effect on activity of acid and alkaline phosphatases. Klose and Tabatabai (2002) obtained similar results for β-glucosidase. Furthermore, studies on phosphatases have shown that the usefulness of toluene is limited in assay procedures that involve only a few hours incubation (Alef and Nannipieri, 1995). We conclude that when short incubation times are used in determining enzyme activity for acid and alkaline phosphatase, β-glucosidase, cellobiohydrolase and chitinase in tropical soils, toluene can be omitted from the assay.

For all enzymes, we observed 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 assimilation of enzymatic reaction products by soil microorganisms (Fig. 3). Both Tabatabai and Bremner (1969); Ajwa et al., 1999 obtained similar results for acid phosphatase. Our results indicate that the method was efficient in determining activities of acid and alkaline phosphatase, β-glucosidase, and chitinase with shorter incubations. A notable exception to the general trend was cellobiohydrolase activity on the clay soil, which showed a significant lag phase. This enzyme displayed very low levels of activity during short incubations, and thus longer incubations and standard curves with lower *p*NP concentrations are required to detect appreciable activity levels. It is unlikely that the low levels of activity represent a true 'lag' phase and that increased activity is associated with microbial growth. The concentrations of *p*NP in the assays were two orders of magnitude below the lowest standard and thus the estimates of enzyme activity were very unreliable.

To answer the question regarding incubation time, we see that, a 2-h incubation time is adequate for the phosphatases. However, for β-glucosidase, cellobiohydrolase, and chitinase, 4 h are required to reach steady product production rates in these African highland soils. β-glucosidase results appear to be very much time dependent in the fallow treatments. For the Teso sites, a break point occurred at 7 h with the relationship between activity and incubation time changing significantly. Prior to this point, the activities increased more or less in parallel. Thus standardizing the incubation to a shorter duration (e.g. 4 h) would avoid problems caused when soils or management effects create conditions, where enzyme activity is not stable within the incubation procedure.

Enzymes are useful indicators of microbial activity because they are sensitive to management practices. Often changes in enzyme activities appear long before other changes, such as organic matter loss. However, the numbers of possible enzymes to measure and the variations in methods to measure them require prior testing to optimize assay application in new settings. The results of this study provide the modifications that expand the applicability of these enzyme activity assays to the degraded tropical soils.

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