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The potential bioavailability of organic C, N, and P through enzyme hydrolysis in soils of the Mojave Desert

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Abstract Increases in the growth rate of plants and microbes in the Mojave Desert in response to predicted increases in precipitation and CO_2 due to global climate change may induce nutrient limitations. This study was designed to measure the pool of potentially bioavailable nutrients in soils of the Mojave Desert. Soils were collected from shrub and interspace microsites and then subjected to amendment with buffered solutions of an excess of various enzymes. The products of each enzyme reaction were then measured and the maximum quantity of hydrolyzable substrates was calculated. In interspace and shrub microsite soils, respectively, 14.5 and 9.7% of the organic C in the form cellulose, 60.0–97.8% and 61.2–100.0% of the organic N in the form protein, and 44.0 and 57.5% of the organic P was hydrolyzable. There were significant differences between microsites for hydrolyzable substrate using all enzyme amendments, except protease. We propose that accumulations of hydrolyzable organic C, N, and P in the Mojave Desert could be a result of the persistently dry soil conditions often found in desert ecosystems and the immobilization of enzymes, which may result in low diffusivity of soil substrates and enzymes and, accordingly, little degradation of organic C, N, and P. Alternatively, rapid nutrient cycling and immobilization by soil microorganisms could account for accumulations of organic C, N, and P. Further refinement of the methods used in this study could lead to a valuable tool for the assessment of potential bioavailability of nutrients in a variety of soils.

Keywords Biogeochemical cycles · Cellulase · Mineralization · Peroxidase · Phosphatase · Protease

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Introduction

Annual net primary production in desert ecosystems is primarily constrained by water limitations (Noy-Meir 1973). However, once water limitations in the desert have been overcome, essential nutrients such as N and P often become the factors that limit primary production. Because inputs of C, N, and P are relatively small to desert ecosystems, recycling of organic-bound nutrients through mineralization is vital.

The N that is present in soils of arid lands is often located in stable organic complexes that

have slow turnover rates (West 1981; Smith et al. 1997). External inputs of N such as deposition and N_2 fixation by microbiotic crusts—growths of algae, lichens, and cyanobacteria on the soil surface—do occur in desert ecosystems. Although inputs of N via N_2 fixation may be significant (Evans and Ehleringer 1993), the N is not necessarily in a form available for uptake by other plants and microbes and must be mineralized first. Bioavailable P largely originates from soil organic and inorganic P pools, both of which can vary widely based on climate and soil properties. In arid and semiarid ecosystems, such as those found in the western United States, often less than half of the total P pool, which can range anywhere from 220 to 1,210 mg P kg^{-1} soil, is present as organic P (Turner et al. 2003). Much of the inorganic P in the soil is either bound in the soil matrix or very insoluble and inaccessible for uptake by organisms (Brady 1990). The organic P fraction, on the other hand, can potentially become available to plants and microorganisms through mineralization.

Carbon is often a limiting nutrient for microbial communities, many of which are involved in nutrient cycling processes. Schaeffer et al. (2003) showed that microbial growth and N transformations could be C limited in Mojave Desert soils. Gallardo and Schlesinger (1992) also showed a significant positive correlation between soil organic carbon content and microbial biomass in desert ecosystems. Organic P and N are also largely covalently bound to carbonaceous material, illustrating the close relationship between carbon mineralization and the availability of other essential nutrients. In fact, lignin and polyphenolic compounds can protect organic N and P from mineralization, slow decomposition, and may cause N and P limitations for plants and microbes (Melillo et al. 1982; Fox et al. 1990).

There have been a number of studies on C, N, and P mineralization processes in arid ecosystems including the effects of water pulses (Austin et al. 2004), depth, microsite, and grazing (Blank 2004), elevated CO_2 levels (Billings et al. 2002; Billings et al. 2004), and disturbance of soils (Bolton et al. 1993) on these biogeochemical processes. Some recent studies have not only investigated the nutrient availability of C, N, and P, but also measured soil enzyme activity as an indirect measurement of mineralization and microbial activity (Li and Sarah 2003; Liu et al. 2000; Green and Oleksyszyn 2002; Blank 2004).

The mineralization of organic compounds in soil is largely a result of enzymatic reactions. Extracellular enzymes may be bound to the outside of cell membranes or released by microorganisms and plants to increase nutrient availability (Burns 1982). After being released into the soil, extracellular enzymes may simply exist in the soil solution or become highly stabilized by bonding with soil colloids or humic substances, associations that can allow enzymes to remain active for many years in some soils (Skujins 1976). Important enzymes involved in the mineralization of C, N, and P in desert soils include cellulase, peroxidase, alkaline phosphomonoesterase, phosphodiesterase, phytase, and protease. Cellulase and peroxidase are involved in the C cycle. The majority of C entering the soil is derived from plant litter, the main component of which is cellulose. Cellulase catalyzes the hydrolysis of cellulose molecules to glucose. The peroxidase enzyme, an enzyme that uses hydrogen peroxide as an oxidant, catalyzes the oxidation of another important sink of C in the soil, phenolic and polyphenolic containing compounds such as lignin and humic acids. Alkaline phosphomonoesterase hydrolyzes the cleavage of monoester phosphate groups in alkaline soils, while phosphodiesterase hydrolyzes the cleavage of phosphodiester bonds, such as those found in cyclic AMP and DNA. Phytase catalyzes the hydrolysis of phytic acid, one of the most abundant organic P compounds in soil, into inositol and phosphoric acid. Proteases catalyze the first step in the mineralization of proteins through hydrolysis of peptide bonds and are rate-limiting enzymes in the N mineralization processes of soil (Ladd and Paul 1973).

Bioavailable C, N, and P in the soil may be considered to consist of two pools: (1) the inorganic pool (in the case of N and P); and (2) the enzyme degradable pool. In standard techniques for measuring the activity of soil enzymes, an excess of substrate specific for the type of enzyme to be measured is added to the soil in a

solution buffered to a pH and adjusted to a specific temperature so as to be at optimal conditions for enzyme activity (Skujins 1976). Excess substrate allows the amount of enzymes in the solution to limit the rate at which the substrate is transformed into a product. By modifying this technique so that an excess of enzymes are added instead of substrate, it should be possible to measure the amount of enzyme degradable substrate present in the soil if the reaction is allowed a sufficient amount of time to reach completion. Using enzymes that are rate limiting in the cycling of specific organic nutrients, a measure of the total pool of bioavailable nutrients can be made.

Measurements of enzyme hydrolyzable P have been carried out by first extracting the soil and then subjecting the extracts to amendment with a variety of phosphatases, often immobilized (Pant et al. 1994a, b; Shand and Smith 1997; He and Honeycutt 2001; Turner et al. 2003; He et al. 2004). Soil extracts have also been hydrolyzed using other immobilized enzymes, such as protease (Warman and Isnor 1989) and arylsulfatase (Whalen and Warman 1996). However, it cannot be assured that the whole bioavailable organic nutrient fraction can be extracted from the soil matrix. Adding an enzyme solution directly to the soil would include insoluble fractions but has rarely been attempted. Tateno (1988) amended soil with protease and cellulase in buffered solutions and measured the products of the enzyme reaction after incubation in an attempt to understand the limiting factors in the degradation of organic matter in the soil. However, beyond the study of Tateno (1988), we know of no other studies that have measured the enzyme hydrolyzable pools of C, N, and P through direct amendment of soils with enzymes. The primary drawback of adding enzymes directly to the soil is that large concentrations of enzymes must be applied since some enzymes may be denatured or deactivated by soil components (Skujins 1976). Also, associations with humic material and lignin may protect much of the substrate from enzymatic degradation (Fuller and Norman 1943; Verma et al. 1975), though it may be possible to overcome this limitation through the use of a mixture of enzymes designed to sequentially degrade the recalcitrant material, followed by the degradation of target substrates.

Our study concentrated on potential bioavailability of soil nutrients in the Mojave Desert. Researchers in forest ecosystems have hypothesized that a progressive N limitation, where bioavailable forms of N are depleted to compensate for increased growth rates of plants in response to high CO_2 levels, might eventually be the primary limiting factor for plant growth (Johnson 2006). Similarly, a progressive nutrient limitation in desert ecosystems due to high CO_2 levels might also occur if water limitations are overcome. Global climate change in the Mojave Desert region has been predicted to cause an increase in summer monsoonal precipitation events (Lioubimtseva and Adams 2004). Thus, it is important to measure the potential pool of bioavailable nutrients in desert soils in order to attempt to predict the degree to which plants and microbes will be able to respond to global climate change.

The objectives of this study were to determine if:

1. there is a large pool of potentially bioavailable (enzyme hydrolyzable) nutrients in Mojave Desert soils.
2. a large portion of the potentially bioavailable nutrients in these soils are protected by lignin and humic acids.
3. there is a large difference in potentially bioavailable nutrients at shrub and interspace microsites in Mojave Desert soils.

Materials and methods

Study site

The study site was located in the Mojave Desert at the US Department of Energy's Nevada Test Site in Nevada, USA, and was a part of the Mojave Global Change Facility, a long-term experiment established to examine the effects of increased monsoon precipitation, increased N-deposition, and soil crust disturbance on this desert ecosystem. The Nevada Test Site provides

high security and has had only minimal disturbance for at least the past 50 years. Most of the annual average of less than 160 mm of rainfall at the site occurs during the winter months, between October and April, in the form of infrequent frontal systems of Pacific origin (Titus et al. 2002). Some precipitation does occur during the summer months, in the form of localized, often intense, thunderstorms that are usually short in duration. The study site is located on a bajada that slopes slightly down to a dry lake bed. The elevation at the site is around 970 m and the range of temperatures is extreme and may fluctuate from a low of -19°C in the winter to up to 48°C in the warmest part of summer.

Perennial shrubs, specifically the C_3 evergreen *Larrea tridentata* and drought-deciduous *Ambrosia dumosa*, dominate the vegetation at the site. Native grasses occur in moderate abundance and cover. Microbiotic crust covered approximately 22–44% of the soil surface of the interspace areas (J. Nadeau, unpubl. data). The microbiotic crust is often made up of algae such as *Microcoleus vaginatus* and *Schizothrix* spp., along with other species of algae that are sometimes closely associated with lichens, such as *Nostoc commune*, *Scytonema hofmannii*, and *Protosiphon cinnamomeus* (Shields and Drouet 1962). Mosses may also be present in these microbiotic crusts, the dominant moss being *Syntrichia caninervis* (Stark et al. 2004), and the relative abundances of algae, lichens, and mosses in these crusts are dependent on the amount of moisture present. Microbiotic crusts have been identified as important contributors to nitrogen fixation and soil stabilization in this desert ecosystem (Belnap et al. 2004).

The soil is a loamy-skeletal, carbonatic, thermic Typic Haplocalcid. Soil texture is a loamy sand in the shallow A1 horizon (0–16 cm), and there is a large spatial heterogeneity in nutrients with an overall average of around 16.0 ± 17.0 and 2.0 ± 2.7 ppm of nitrate and ammonium, respectively, 15 ± 9 ppm of bicarbonate extractable phosphorus, and soil organic matter concentrations of around 0.88 ± 0.45% (Titus et al. 2002). Soil bulk densities averaged around 1.34 g cm^{-3}, with a collection of finer soils beneath shrubs as a result of aeolian dust transport.

Soil sampling

Three soil samples of approximately 436.5 cm^3 were collected randomly from six plots using a soil core to a depth of about 10 cm. Sampling depth was chosen based on visual observations of the A1 horizon depth in the plots, representing the zone of maximum organic matter accumulation. Three plots each were located in areas dominated by microbiotic crusts (interspace) and in areas dominated by the shrub, *L. tridentata*. The soil from each core was stored in sealed plastic bags that were kept in the shade until being transported to the lab. In the lab, the <2-mm fraction of each soil core was obtained and roots removed by passing the soil through a sieve and manually removing any remaining roots. Each of the three samples from each microsite was then separately homogenized, air-dried, and reserved for analyses.

Incubation time and enzyme concentration

Incubations of 1–4 days of the soil samples from each of the three replicate shrub plots were carried out in order to assess the effects of incubation time on the amount of substrate hydrolyzed. The incubation time trials were done for cellulase using an enzyme concentration of 18.2 units ml^{-1}, phosphatase using an enzyme concentration of 40 units ml^{-1}, and protease with an enzyme concentration of 20 units ml^{-1}. The enzyme concentrations used for each of the time trials were chosen to be high, based on Tateno (1988) and Turner et al. (2003) and given our limited supplies of enzymes and the number of samples to be tested, to insure maximum hydrolysis.

Concentrations of approximately 4.5, 9.1, 13.6, 18.2, and 22.7 units ml^{-1} of cellulase; 10, 20, 30, 40, and 50 units ml^{-1} of phosphatase; and 5, 10, 15, and 20 units ml^{-1} of protease were tested in an attempt to fit the data to an asymptotic model and to assure maximum organic matter degradation by varying the amount of the stock cellulase, phosphatase, and protease solution added to each sample but at the same time maintaining the total volumes described below for each enzyme amendment. All incubations were carried out

for 24 h based upon the results of the incubation time trials.

Cellulase amendment

A stock cellulase solution containing 50 units ml^{-1}, based on the percentage used by Tateno (1988), was produced by dissolving a crude cellulase powder (Sigma, from *Trichoderma viride*) in a 2-M acetate buffer solution at pH 5.5. To 2 g of soil, 0.2 ml of toluene to suppress microbial activity was added followed by the addition of a specific concentration of a cellulase solution produced from the stock solution described above. The total amount of all solutions added to the soil was kept constant at 11.2 ml for all experiments, excluding the addition of peroxidase in which the volume of all solutions was kept constant at 12.2 ml. After the addition of the cellulase solution, the samples were incubated in a shaking hot water bath set at 50°C for 24 h. After incubation, the samples were centrifuged and the supernatant analyzed for glucose using the 3,5-dinitrosalicylic acid method (Miller 1959). Results are presented in mg glucose g^{-1} dry soil.

Phosphatase amendment

A stock phosphatase solution containing 50 units ml^{-1} of alkaline phosphomonoesterase, 10 units ml^{-1} phytase, and 2 units ml^{-1} phosphodiesterase (based on approximately 20–50 times the concentrations used by Turner et al. (2003) in order to compensate for inactivation of enzymes in the soil matrix) was produced by dissolving a lyophilized powder of alkaline phosphomonoesterase (Sigma, Type VII-S, from bovine intestinal mucosa), a crude phytase powder (Sigma, from *Aspergillus ficuum*), and a powder of crude dried venom containing phosphodiesterase I (Sigma, Type IV, from *Crotalus atrox*) in a 0.5-M $NaHCO_3$ buffer at pH 7.2 containing 2 mM $MgCl_2$. To 1 g of fine soil, 0.2 ml of toluene was added followed by the addition of a specific concentration of a phosphatase solution produced from the stock solution described above. The total amount of all solutions added to the soil was kept constant at 5.2 ml for all experiments, excluding the addition of peroxidase in which the volume of all solutions was kept constant at 6.2 ml. After the addition of the phosphatase solution to the soil, the samples were incubated in a shaking hot water bath set at 37°C for 24 h. Following incubation, the samples were centrifuged and the supernatant removed and stored in a freezer until an orthophosphate analysis was conducted. The remaining soil was then extracted with 4 ml of 1 M HCl and the HCl extracts were stored in a freezer until an orthophosphate analysis was conducted. Orthophosphate analysis on the samples was accomplished colorimetrically using the molybdate-blue/ascorbic acid method after 1 ml of a 5% sodium dodecyl sulfate solution was added to 1 ml of sample followed by dilution with nanopure water up to a total volume of 10 ml. Results are presented in μg ortho-P g^{-1} dry soil.

Protease amendment

A stock protease solution containing 50 units ml^{-1}, based on approximately two times the percentage of enzyme used by Tateno (1988), was produced by dissolving a powder of protease (Sigma, Type XIV, from *Streptomyces griseus*) in a 50-mM Tris buffer at pH 7.6. To 2 g of fine soil, 0.3 ml of toluene was added followed by the addition of a specific concentration of a protease solution produced from the stock solution described above. This protease contains a variety of endo- and exopeptidases and is capable of the nearly complete hydrolysis of proteins. The total amount of all solutions added to the soil was kept constant at 10.3 ml for all experiments. After the addition of the protease solution to the soil, the samples were incubated in a shaking hot water bath set at 37°C for 24 h. Following incubation, samples were centrifuged and 5 ml of a 15% (w/v) trichloroacetic acid solution was added to a 5-ml aliquot of the supernatant in order to precipitate the proteins. The samples were centrifuged again following protein precipitation and the supernatant analyzed for amino acids using a Bio-Rad DC protein assay based on the Lowry protein assay. Results are presented in mg tyrosine g^{-1} dry soil.

Assuring an excess of active enzyme after incubation

To assure that the enzymes were still active in the soil at the end of the incubations, trials were done in which toluene and the cellulase, phosphatase, and protease solutions were added to the soil followed by a 24-h incubation as described above. After 24 h, substrate was added in the form of a 7% cellulose (Sigma, Type 101) in a 2-M acetate buffer at pH 5.5 for cellulase amendments and a 2% (w/v) casein solution in a 50-mM Tris buffer at pH 7.6 for protease amendments to bring the total volume of solutions added up to the levels described above, after which the samples were then incubated again for 24 h. After the second incubation, the samples were centrifuged and analyzed as described above. For phosphatase, after the 24-h incubation period, the samples were analyzed for alkaline phosphomonoesterase activity using a modified version of the method described by Tabatabai and Bremner (1969) and Elvazi and Tabatabai (1977). Briefly, a buffer of 0.5 M $NaHCO_3$ at pH 7.2 was used in substitution of modified universal buffer at a pH of 11. Following incubation and addition of reagents, the samples were filtered and the absorbance of the resulting filtrate was analyzed at 420 nm on a spectrophotometer. The alkaline phosphomonoesterase activity was expressed in terms of μg *p*-nitrophenol g^{-1} of dry soil per hour.

Peroxidase amendments

To test whether much of the soil cellulose and organic P substrates were protected through association with lignin and humic acids, a mixture of cellulase or phosphatase and peroxidase enzymes was produced by adding a lyophilized powder of peroxidase (Sigma, from *Arthromyces ramosus*) to the stock cellulase or phosphatase solution to produce a solution containing 50 units ml^{-1} cellulase and 10 units ml^{-1} peroxidase or 50 units ml^{-1} of alkaline phosphomonoesterase, 10 units ml^{-1} phytase, 2 units ml^{-1} phosphodiesterase, and 10 units ml^{-1} peroxidase. The procedure for enzyme amendment using this cellulase or phosphatase/peroxidase solution was the same as described above except that 1 ml of a 4-mM H_2O_2 solution was added to the reaction mixture for a total solution volume of 12.2 ml for cellulase amendments and 6.2 ml for phosphatase amendments.

Controls

Controls of all amendments were carried about by (1) adding all solutions without soil present and (2) adding all solutions with soil but inhibiting enzyme activity by the addition of 1 ml of 5 mM phenylmercuric acetate for cellulase and phosphatase amendments or precipitating proteins immediately with a 15% (w/v) trichloroacetic acid solution for protease amendments. Phenylmercuric acetate was found to be an adequate inhibitor of cellulase and phosphatase activity in pre-experiment trials (data not shown).

Total and organic C, N, and P

Soils were analyzed for organic C content by first treating the soil with sulfurous acid followed by organic C measurements on a PerkinElmer Series II CHNS/O Auto-analyzer. Total and organic N contents were also determined using the PerkinElmer Series II CHNS/O Auto-analyzer. Total and organic P concentrations were determined using the method described by Bowman (1989).

Calculations of potential bioavailability

To calculate the total amount of substrate present in the soil that could be acted upon by the enzyme, we estimated an asymptotic value of the total amount of product formed as the enzyme concentration was increased. This approach is similar to that used in determinations of potentially mineralizable N (PMN) and C in incubations in which the accumulation of mineralized product is found to approach an asymptote representing the total mineralizable substrate (Molina et al. 1980). This approach uses a fit to the asymptotic equation:

$$y = a\left(1 - e^{(-bx)}\right) + c$$

where "*y*" is the amount of product formed, "*x*" is the enzyme concentration, "*c*" is the *y*-inter-

cept, ''*b*'' is a parameter defining the degree of curvature leading to the asymptote, and ''*a*'' is the asymptote. The variable ''*c*'' was set to 0 for the cellulase and phosphatase amendments since a lack of an enzyme amendment should theoretically lead to the formation of little or no product. For protease amendments, the variable *c* was allowed to be less than 0 as a result of an observed minimum addition of enzyme necessary to produce any product, likely caused by inactivation of the enzyme by the soil.

For cellulase amendments, in which glucose was the end product, the mass of the maximum amount of product formed was divided by a factor of 2.5 based on the C content of glucose to convert to mg C g^{-1} soil in the form of cellulose. The maximum amount of product formed in phosphatase amendments, ortho-P, was not transformed in any way since it could be directly related to total and organic P concentrations. Finally, the end product of the protease amendment, as measured by the Bio-Rad DC protein assay (Lowry method) in mg tyrosine g^{-1} soil, was converted to mg N g^{-1} soil using a conversion factor of 1.54–2.54, which was based on calculations of Lowry reactive amino acid concentrations in desert soils (Martens et al. 2006) and an average N content of amino acids in soil of 13.2% (McClain and Martens 2005).

Statistical analyses

SPSS 14.0 (SPSS Inc., Chicago, IL, USA 2005) was used for all statistical tests. Each enzyme amendment was analyzed using a one-way ANOVA with the amount of product formed as the dependent variable and fixed factors of microsite (adjacent to shrub, interspace) and concentration (9.1 and 13.6 units ml^{-1} cellulase; 20 and 30 units ml^{-1} phosphatase; 10 and 15 units ml^{-1} protease). Each enzyme and enzyme combination was analyzed separately and the two concentrations analyzed for each enzyme amendment were chosen to approximate the concentrations immediately before the concentrations at which the amount of product formed reached a maximum in the asymptotic model. Data were fit to the asymptotic model described above using a non-linear regression in SPSS 14.0 with the amount of product formed as the dependent variable. Finally, for each time trial, the amount of product formed was analyzed using a repeated measures ANOVA with day (time) as the within-subjects factor. All data met the assumptions for normality and Mauchly's sphericity for all statistical analyses described above and, therefore, no transformations were necessary.

Results

Incubation time trial and enzyme concentration effects

An incubation of 24 h appeared to be sufficient for an optimum hydrolysis of cellulose, organic P, and protein (Fig. 1a–c). There were no significant day-to-day variations according to a repeated measures ANOVA.

Both shrub and interspace microsite soils amended with cellulase had an increase in glucose hydrolyzed from cellulose with increasing enzyme concentration until a maximum was reached, after which glucose concentrations remained relatively constant, corresponding well with the asymptotic model (Fig. 2a). Shrub soils had lower levels of hydrolyzed cellulose than interspace soils when amended with cellulase alone, starting at 0.31 mg glucose g^{-1} soil at an enzyme concentration of 4.5 units ml^{-1} and approaching a maximum at 0.78 mg glucose g^{-1} soil at 13.6 units ml^{-1}. Interspace soils amended with cellulase alone produced 0.78 mg glucose g^{-1} soil at an enzyme concentration of 4.5 units ml^{-1} and reached a maximum at 1.39 mg glucose g^{-1} soil at 18.2 units ml^{-1}. A comparison of the 9.1- and 13.6-unit ml^{-1} enzyme concentrations for interspace (Fig. 3a) and shrub (Fig. 3b) microsites revealed that: (1) interspace soils released significantly more glucose upon amendment with cellulase than shrub soils, (2) significantly more glucose was produced as enzyme concentration was increased, and (3) interspace soils had a significantly higher amount of glucose released compared to shrub soils as the concentration of enzymes amended to the soil increased (Table 1). Calculated asymptotes for cellulase amendments

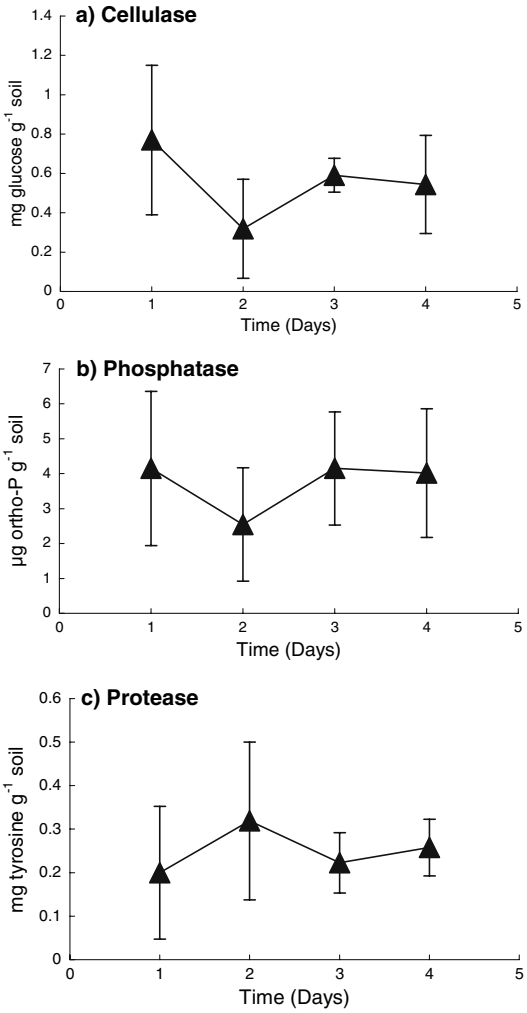

Fig. 1 Time trial for enzyme additions of 18.2 units ml^{-1} cellulase (**a**), 40 units ml^{-1} phosphatase (**b**), and 20 units ml^{-1} protease (**c**) to three soils from shrub microsites. Data points are the means and the *error bars* represent standard deviations ($n = 3$). Repeated measures ANOVA showed no significant differences over time

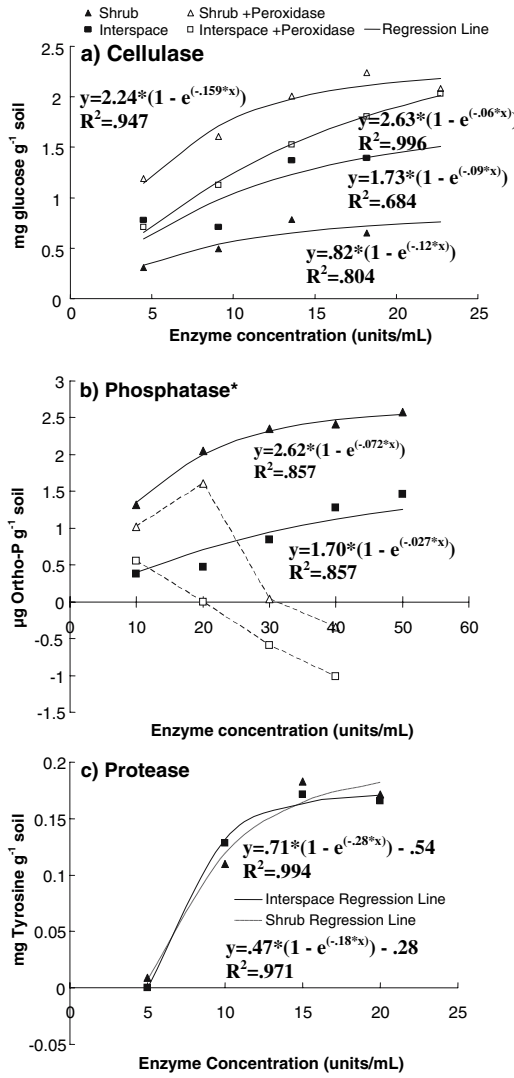

Fig. 2 Dynamics of enzyme concentration effects on the amount of potentially bioavailable substrate degraded and calculated regression lines for (**a**) cellulase, (**b**) phosphatase, and (**c**) protease. (▲) denotes shrub microsite with enzyme addition, (■) interspace microsite with enzyme addition, (△) shrub microsite with enzyme and peroxidase addition, and (□) interspace microsite with enzyme and peroxidase. *phosphatase enzyme concentrations represent alkaline phosphomonoesterase concentrations, although the enzyme solution was a mixture of phosphatases (10 units ml^{-1} phytase and 2 units ml^{-1} phosphodiesterase at the 50-unit ml^{-1} alkaline phosphomonoesterase concentration)

alone were 1.73 ± 0.82 and 0.82 ± 0.25 mg glucose g^{-1} soil for interspace and shrub soils, respectively.

Increasing phosphatase enzyme concentrations led to increased ortho-P concentrations in both shrub and interspace microsite soils, again corresponding well with the asymptotic model (Fig. 2b). The interspace soil had lower levels of ortho-P hydrolyzed at all enzyme concentrations than the shrub soil, ranging from a low of 0.38 μg ortho-P g^{-1} soil at the lowest phosphatase concentration tested to a high of 1.46 μg ortho-P g^{-1} soil at the highest enzyme concentration. The shrub soil had a low of 1.31 μg ortho-P g^{-1} soil at the lowest phosphatase concentration and a

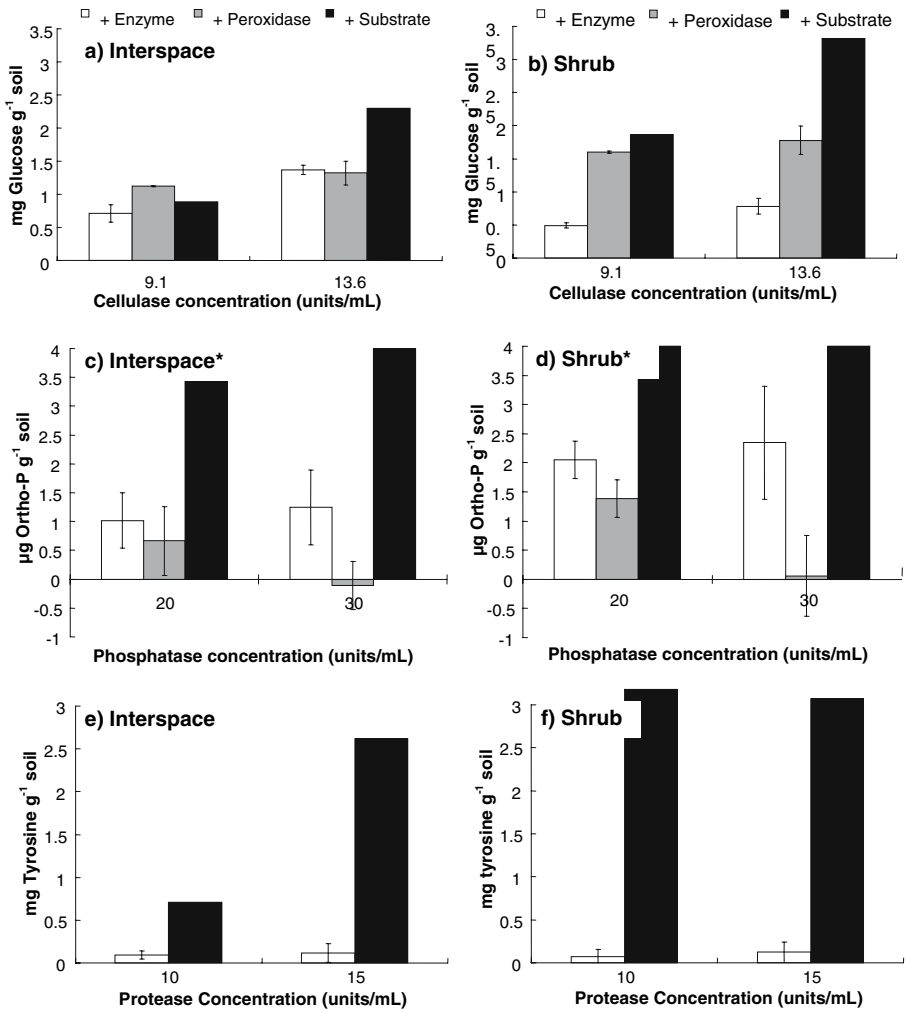

Fig. 3 A comparison of two enzyme concentrations on microsite soil substrate degradation and the effects of excess soil substrate addition to the soil after a 24-h enzyme incubation for amendments of cellulase (**a**, **b**), phosphatase (**c**, **d**), and protease (**e**, **f**). + Enzyme (*white*) denotes enzyme addition, + Peroxidase (*gray*) denotes enzyme and peroxidase addition, and + Substrate (*black*) denotes a 24h enzyme incubation period followed by excess substrate addition. *Error bars* represent standard deviations (n = 3). * Phosphatase substrate addition produced too much hydrolyzed substrate to be measured accurately

high of 2.56 ug ortho-P g^{-1} soil at the highest phosphatase concentration. A comparison of the 20- and 30-unit ml^{-1} alkaline phosphomonoesterase concentrations for interspace (Fig. 3c) and shrub (Fig. 3d) microsites illustrated there was a significant difference in the amount of product formed between interspace and shrub microsites (Table 1). Calculated asymptotes for the phosphatase enzyme mixture were 1.70 ± 0.90 and 2.62 ± 0.05 μg ortho-P g^{-1} soil for interspace and shrub soils, respectively.

Increasing the concentration of protease led to increased amounts of protein hydrolyzed in both interspace and shrub microsites until a maximum of 0.16 mg tyrosine g^{-1} soil and 0.17 mg tyrosine g^{-1} soil was reached for the interspace and shrub soils, respectively, at an enzyme concentration of 15 units ml^{-1}, fitting well with the asymptotic model (Fig. 2c). The lowest amount of hydrolyzed protein, representing approximately 0 mg tyrosine g^{-1} soil, occurred at the 5-unit ml^{-1} enzyme concentration for both the interspace and

Table 1 ANOVA *F* and *P* values for a comparison of two microsites (interspace; adjacent to shrub) and two enzyme concentrations (9.1 and 13.6 units ml^{-1} cellulase, 20 and 30 units ml^{-1} phosphatase, 10 and 15 units ml^{-1} protease) for each enzyme amendment ($n = 3$)

Enzyme amendment	Microsite (*M*)		Enzyme concentration (*C*)		*M* × *C*	
	F	*P-value*	*F*	*P-value*	*F*	*P*-value
Cellulase	50.40	<0.001**	69.66	<0.001**	10.72	0.011*
Cellulase and peroxidase	34.19	<0.001**	5.51	0.047*	0.01	0.907
Phosphatase	8.06	0.022*	0.482	0.507	0.01	0.928
Phosphatase and peroxidase	11.96	0.009**	2.17	0.179	0.81	0.393
Protease	0.01	0.908	0.43	0.529	0.07	0.799

A microsite (*M*) and enzyme concentration (*C*) interaction, denoted by $M \times C$, indicates that there is a larger enzyme amendment effect on product formation in one of the microsites with changing enzyme concentrations

*Significant at $P \leq 0.05$

**Significant at $P \leq 0.01$

shrub soils. A comparison of the 10- and 15-unit ml^{-1} protease concentrations for interspace (Fig. 3e) and shrub (Fig. 3f) microsites illustrated no significant differences between interspace and shrub microsites as well as both enzyme concentrations (Table 1). Calculated asymptotes for the protease enzyme mixture were 0.71 ± 0.24 and 0.47 ± 0.21 mg tyrosine g^{-1} soil and *y*-intercepts of -0.54 ± 0.24 and -0.28 ± 0.24 mg tyrosine g^{-1} soil for interspace and shrub soils, respectively.

Peroxidase amendments

Treatments with a mixture of cellulase and peroxidase greatly increased the hydrolysis of cellulose. When shrub and interspace soils were amended with a mixture of cellulase and peroxidase, shrub soils consistently had higher levels of glucose produced, starting at 1.19 mg glucose g^{-1} soil at an enzyme concentration of 4.5 units ml^{-1} and peaking at 2.08 mg glucose g^{-1} soil at 18.2 units ml^{-1}. Interspace soils amended with cellulase and peroxidase did not seem to fully peak at the highest enzyme concentration used but produced 0.71 mg glucose g^{-1} soil at an enzyme concentration of 4.5 units ml^{-1} and reached a concentration of 2.03 mg glucose g^{-1} soil at 22.7 units ml^{-1}. Calculated asymptotes for cellulase and peroxidase amendments were 2.63 ± 0.15 and 2.24 ± 0.11 mg glucose g^{-1} soil for interspace and shrub soils, respectively. Amendments with both cellulase and peroxidase yielded significant differences between microsites and enzyme concentrations, but no significant interaction between microsite and enzyme concentration (Table 1).

Increasing the concentration of a mixture of phosphatase and peroxidase enzymes actually led to a decrease in ortho-P concentrations over all enzyme concentrations for the interspace soil and a consistent decrease after an initial increase in ortho-P concentrations for the shrub microsite soil (Fig. 2b), possibly indicating some form of interference at higher enzyme concentrations. The shrub microsite soil also had greater ortho-P concentrations than the interspace soil at all enzyme concentrations when a mixture of phosphatase and peroxidase enzymes was used. The maximum ortho-P hydrolyzed using the phosphatase and peroxidase mixture, 1.61 μg ortho-P g^{-1} soil, occurred at the 20-unit ml^{-1} alkaline phosphomonoesterase concentration. The interspace soil, on the other hand, had a maximum of 0.55 μg ortho-P g^{-1} soil at the lowest enzyme concentration. In both soils higher concentrations of enzymes actually resulted in ortho-P concentrations lower than the control.

Substrate additions following 24-h incubations

Additions of standard substrates to soils after a 24-h incubation with the enzyme amendments showed that all three enzyme mixtures still had substantial activity after incubation. Additions of cellulose to the soil following incubation of soil and enzyme mixtures for 24 h show substantially higher glucose concentrations from hydrolyzed cellulose at the 13.6-unit ml^{-1} enzyme concentra-

tion for both interspace (Fig. 3a) and shrub (Fig. 3b) microsites, although at the 9.1-unit ml^{-1} enzyme concentration the levels of glucose were only slightly higher in shrub soils and approximately the same for interspace soils. Additions of *p*-nitrophenyl phosphate to the soil following incubation of soil and enzyme mixtures for 24 h led to the production of nitrophenol in concentrations too great to be measured in both interspace (Fig. 3c) and shrub (Fig. 3d) microsites, indicating that the phosphomonoesterase was still active after 24 h. Finally, additions of casein to the soil following incubation of soil and the protease solution for 24 h led to substantially higher production of Lowry reactive amino acids in both interspace (Fig. 3e) and shrub (Fig. 3f) microsites, indicating the protease enzymes were still active. There was a large increase in mg tyrosine g^{-1} soil for interspace soils and essentially no difference in shrub soils between the 10- and 15-unit ml^{-1} protease concentrations.

Potential bioavailability of C, N, and P

The measured levels of organic C in soils adjacent to the interspace and shrub microsite soils used in the enzyme amendments were higher in the shrub than interspace soils, while the calculated percentage of organic C corresponding to the maximum hydrolyzable substrate from cellulase and peroxidase amendments was higher in interspace than shrub soils (Table 2). The total N and organic N levels measured in soils adjacent to the interspace and shrub microsite soils used in the enzyme amendments were again higher in shrub than interspace soils (Table 2). The calculated percentage of organic N that is potentially bioavailable based upon the maximum hydrolyzable substrate from protease amendments is dependent on the assumptions of relating the tyrosine standard to the total N in the form of hydrolyzed amino acids. The ranges based on the range in the conversion factor were also higher in shrub than interspace soils (Table 2). Finally, the total P measured in interspace and shrub microsite soils used in the enzyme amendments were approximately the same for interspace and shrub soils while the organic P levels were higher for shrub than interspace soils (Table 2). The calculated percentage of organic P that is potentially bioavailable based upon the maximum hydrolyzable substrate from phosphatase amendments was also higher in shrub than interspace soils. Because the soils contained more inorganic P than organic the percentages were much lower, but still higher in shrub than interspace soils, when expressed as a percentage of total P (Table 2).

Discussion

Enzyme amendments

Amending Mojave Desert soils with enzymes involved in C, N, and P mineralization suggests that a large portion of the non-labile organically bound C, N, and P could potentially become bioavailable. A majority of organically bound P is hydrolyzable in Mojave Desert soils, whereas much of the C was not subject to enzymatic attack by cellulase. Although there were uncertainties associated with equating the release of Lowry reactive amino acids with the amount of hydrolyzed N released by protease, our calculated range of N released from protein hydrolysis suggests that much of the organically bound N is bioavailable.

Given that the largest portion of organic C entering the soil is in the form of plant residues, and that cellulose is the most abundant component of these plant residues (Paul and Clark 1996), one would expect that a large fraction of the organic C in the soil would be released during amendment with cellulase. In contrast, less than 10% of the organic C pool in Mojave Desert soils was released in the form of reducing sugars as a result of amendment with cellulase alone, suggesting that the easily degradable cellulose fraction may not be a major constituent of soil organic matter. In fact, Gregorich et al. (1994), asserts that greater than 75% of soil organic matter is resistant to degradation in agricultural soils. Other researchers using incubation methods to measure potentially mineralizable C (release of respired CO_2) have found similar results (Scott 1998; Karbozova-Saljnikov et al. 2004). Tateno (1988), in a study using soil amendments with

Table 2 Total, organic, and calculated potentially bioavailable (PB) C, N, and P for interspace and shrub microsites

Microsite	Nutrient	Total	Organic	PB	Organic PB (%)	Total PB (%)
Interspace	Carbon (mg g^{-1} soil)	–	7.26 ± 0.06[a]	1.05 ± 0.06[b]	14.5	–
	Nitrogen[c] (mg g^{-1} soil)	0.46 ± 0.01[a]	0.45 ± 0.01[a]	0.27–0.44	60.0–97.8	58.7–95.6
	Phosphorus (μg g^{-1} soil)	50.96 ± 0.52[a]	3.86 ± 0.26[a]	1.70 ± 0.90[b]	44.0	3.3
Shrub	Carbon (mg g^{-1} soil)	–	9.25 ± 0.16[a]	0.90 ± 0.04[b]	9.7	–
	Nitrogen[c] (mg g^{-1} soil)	0.50 ± 0.01[a]	0.49 ± 0.01[a]	0.30–0.49	61.2–100.0	60.0–98.0
	Phosphorus (μg g^{-1} soil)	49.82 ± 2.54[a]	4.56 ± 2.27[a]	2.62 ± 0.05[b]	57.5	5.6

[a] Mean ± S.D. (n = 6 for interspace microsites, n = 3 for shrub microsites)

[b] Standard deviations based on the standard deviation of the estimate of the asymptote (a) from Fig. 2 converted into units of C, N, or P

[c] Potentially bioavailable N calculated based upon a conversion factor of 1.54–2.54 mg N mg^{-1} Lowry reactive amino acid in the soil

cellulase, found only about 7.2% of the soil organic matter to be hydrolyzable cellulose. The association of cellulose with lignin, especially in plant residues originating from woody material, can protect cellulose from degradation by enzymes (Fuller and Norman 1943; Grabber 2005). Our results seem to coincide well with this observation because we had a relatively large increase in reducing sugars produced, approximately 14.5% of soil organic C, when soils were amended with an enzyme mixture consisting of cellulase and the lignin-degrading enzyme, peroxidase. To our knowledge, this is the first attempt to characterize the bioavailability of cellulose using a mixture of cellulase and peroxidase enzymes. There is some uncertainty regarding the origin of the increase in reducing sugars because a portion may have been released from humic and tannin-like complexes, many of which include a carbohydrate moiety, as a result of degradation by the peroxidase enzyme. Controls consisting of additions of peroxidase alone to the soil could help to alleviate some of this uncertainty.

The majority of soil P in the Mojave Desert is in the acid-soluble inorganic form, likely in a biologically unavailable calcium phosphate form similar to other desert soils (Lajtha and Schlesinger 1988). Our Mojave Desert soils have low levels of organic P, characteristic of desert soils in general (Turner et al. 2003). The organic P fraction is largely bioavailable since greater than 50% of the organic P was hydrolyzed to ortho-P after amending the soil with a mixture of phosphatases. Turner et al. (2003), who exposed bicarbonate soil extracts to enzyme hydrolysis, found that between 37 and 87% of the organic P was hydrolyzable. Other researchers, using similar methodology, found between 1 and 100% of the bicarbonate extractable P to be enzyme hydrolyzable (Otani and Ae 1999; Hayes et al. 2000; He et al. 2004). He et al. (2004) did a sequential extraction with H_2O, bicarbonate, and NaOH on three soils, finding that 26–66, 84–100, and 29–49% of the organic P in each extract, respectively, was enzyme hydrolyzable. Our study, in which phosphatase enzymes were amended directly to the soil, may have better represented the entire organic P fraction since these other studies exclusively examined enzyme hydrolysis of extractable organic P. Consequently, our results generally agree with the findings of other researchers that a large portion of the soil organic P is potentially hydrolyzable.

In contrast to the increased hydrolysis of cellulose or cellulose-like compounds in the soil as a result of addition of a mixture of cellulase and peroxidase, though, phosphatase did not seem to have any increased hydrolysis when combined with peroxidase. Some possible interference may account for the low levels of phosphate hydrolyzed during amendment with phosphatase and peroxidase, since the levels of phosphate hydrolyzed were actually less than the controls at high levels of peroxidase. A possible explanation of this interference may be that the combination of peroxidase and phosphatase increased the availability of phosphate adsorption sites in the soil. Nonetheless, the data for phosphatase and peroxidase amendment at low

enzyme concentrations suggests little to no effect of peroxidase on the amount of hydrolyzed phosphate. Thus, very little of the organic P may be protected by associations with lignin or humic materials in Mojave Desert soils.

The organic N pool in Mojave Desert soils, much like the organic P pool, seems to be largely potentially bioavailable. The majority of the total N pool in the Mojave Desert, like most other soils, is bound in soil organic matter. In previous studies, most soils have been shown to have any where from 7 to 50% of the soil organic N in the form of monomeric or polypeptide amino acids (Stevenson 1982). Using ^{15}N-NMR, other studies have confirmed that up to 87% of soil N may be in the form of amide bonds such as those found between peptides (Knicker and Hatcher 1997). Research conducted on an agricultural soil has shown that greater than 60% of the soil organic N can be present in the form of amino acids (Martens et al. 2006). Our findings suggest that anywhere from 60 to 100% of the soil organic N is in the form of enzyme hydrolyzable proteins in Mojave Desert soils, although there are large uncertainties associated with our calculations of enzyme hydrolyzable organic N. This range indicates the possibility for much higher amino acid and protein contents in Mojave Desert soils, as a percentage of total soil N, than other researchers have previously reported for other soils (Paul and Clark 1996). El Gharous et al. (1990) found that only up to 36% of the total soil N in arid and semiarid soils was PMN using incubation and hyperbolic extrapolation techniques, whereas using the more widely used exponential model they reported the PMN to range from 7 to 22% and to be on the same order as results found by other researchers. Additional evidence that points to an overestimation of PMN in our study is that other researchers have found PMN to range from 0.01 to 0.31 mg N g^{-1} soil in the USA (Benbi and Richter 2002) and, more specifically, 0.04–0.10 mg N g^{-1} soil in Mojave Desert soils (Billings et al. 2004) using incubation and extrapolation techniques, whereas our results suggest a range 0.27–0.49 mg g^{-1} soil in the Mojave Desert. Uncertainties about our calculated hydrolyzable organic N content of Mojave Desert soils arise from issues concerning the conversion of Lowry reactive amino acid N content to total soil protein N content. The study also showed that the use of controls for autolysis of protease enzyme is necessary when attempting to measure hydrolyzable N in the soil.

Attempts to ascertain whether proteins were protected by associations with lignin or humic material in the soil through amendments of a mixture of protease and peroxidase showed a large increase in the amount of amino acid released (data not shown). We determined this data was unreliable because it represented a release of amino acid N that was not feasible based on our measurements of total and organic N in interspace and shrub soils. We concluded that the excess amino acids released may have been a result of protease enzymes degrading the excess of amended peroxidase enzymes in the soil (Dosoretz et al. 1990). Sequential amendments of each enzyme and a simple control combining protease and peroxidase enzyme solutions without soil would help to alleviate this issue, but we determined this to be an unnecessary step in this study because amendments of protease alone already produced nearly maximum organic N degradation in our soils, indicating that very little, if any, protein is protected by associations with lignin or humic material in Mojave Desert soils.

Microsite effects

The higher levels of organic nutrients found in this study in shrub microsites is not surprising given the well documented phenomenon of the ''fertile islands'' in desert ecosystems (Schlesinger et al. 1990; Whitford et al. 1997; Titus et al. 2002). Interestingly, there were significant differences between shrub and interspace microsite soils for enzyme hydrolyzable substrate when cellulase, cellulase and peroxidase, and phosphatases were added, but not when protease was added. As was expected, there was more enzyme hydrolyzable organic P in shrub microsite soils. The lack of a significant difference in hydrolyzable protein concentration between microsites was surprising given that soils near shrubs have higher productivity than interspace soils due to the aforementioned ''fertile island'' effect. The percentage of organic N based on total N was not greatly

different between microsites either, although shrub microsite soils did have slightly higher N concentrations. A possible explanation for similar protein contents between soils may be that the microbiotic crust organisms present in the interspace areas, though less productive than organisms in shrub areas, may have access to N from N_2 fixation by cyanobacteria (Evans and Ehleringer 1993), possibly allowing these organisms to produce more nitrogen rich organic compounds. Another possible consideration is that a higher percentage of the N in the shrub area soils is incorporated into humic substances.

Contrary to our expectations, interspace microsite soils had higher levels of potentially bioavailable cellulose when amended with cellulase alone, although a mixture of cellulase and peroxidase produced comparable levels of potentially bioavailable cellulose in shrub and interspace microsite soils. However, the large response in the amount of reducing sugars released in shrub microsites, from about 3.5% to greater than 9%, as a result of amendment with both cellulase and peroxidase, could indicate that cellulose in shrub microsite soils is more protected by associations with lignin. Higher degrees of cellulose protection in shrub soils may be a result of the greater amounts of cellulose derived from woody tissue with high lignin contents, whereas much of the cellulose in interspace regions may originate from grasses, leaf, and root litter.

Methodological considerations

Manipulations of incubation time and enzyme concentration indicated that 24-h incubations were sufficient for complete substrate hydrolysis in the soil at high enzyme concentrations for all enzyme amendments. Enzyme amendments to the soil essentially followed an asymptotic model for the accumulation of products by enzymatic hydrolysis, with increasing enzyme concentrations leading to a maximum level of product formation. Once enzyme limitations were overcome, the limiting factor of the enzymatic reaction became the substrate concentration, making it possible to measure the maximum potentially bioavailable substrate present in these soils. Protease amendments at low concentrations produced little to no substrate hydrolysis, possibly implying that these enzymes were becoming deactivated in the soil at low concentrations. Additions of excess substrate to the soils after 24-h incubations with enzyme amendments showed that the enzymes were still active at higher enzyme concentration amendments for all enzymes.

Due to the paucity of studies that have attempted direct amendment of enzymes to the soil to measure enzyme hydrolyzable substrate, our methods were relatively novel and could use further refinement. Nonetheless, the data collected in this study suggests that there is great potential for measurements of potential bioavailability using enzyme amendments. We recommend further research in this field using other enzymes involved in mineralization, sequential enzyme amendment using a combination of enzymes designed to obtain full hydrolysis of mineralizable substrate, and the application of these methods to different soil types.

Conclusions

Enzyme amendments to the soil in high concentrations for a period of time sufficient for maximum hydrolysis of substrate were found to be an adequate method to estimate the potentially bioavailable pools of C, N, and P. Our results suggest that the majority of the soil organic N and P and a good portion of the soil organic C is potentially bioavailable in the Mojave Desert. Higher potential bioavailability of P was found in shrub microsite soils, while there was no significant difference found in the degree of potential bioavailability of N between interspace and shrub microsite soils. Carbon, in the form of cellulose, was found to be more bioavailable in interspace soils and our results suggest a higher degree of protection for cellulose in shrub microsite soils compared to interspace. The reasonably large percentages of potentially bioavailable C, N, and P leads to questions concerning why there are accumulations of organic C, N, and P in Mojave Desert soils. We propose that these accumulations could be a result of the immobilization of enzymes along with the persistently dry soil conditions often found in desert ecosystems,

which may result in low diffusivity of soil substrates and enzymes and, accordingly, little degradation of organic C, N, and P. It is also possible that substrates can be protected in nanopores from enzymatic attack. Alternatively, rapid nutrient cycling and immobilization by soil microorganisms may maintain much of soil C, N, and P in hydrolyzable organic forms, although this seems unlikely given the small amount of microbial biomass present in Mojave Desert soils (Billings et al. 2004). Such explanations could be a valuable insight into the poorly understood phenomenon of sequestration in desert ecosystems. Further refinement of the methods used in this study would be beneficial for determining the pools of potentially bioavailable nutrients in a variety of soils. Also, enzyme amendments with enzymes specific for particular substrates may lead to better understandings of the nature of the potentially bioavailable nutrient pools.

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