# Chapter 6
# Soil Enzyme Activities as Affected by Manure Types, Application Rates, and Management Practices

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**Abstract** Manure application can restore soil ecosystem services related to nutrient cycling and soil organic matter (SOM) dynamics through biochemical transformations mediated by soil enzymes. Soil enzymes are crucial in soil metabolic functioning, as they drive the decomposition of organic residues, humification processes, transformations leading to the release of plant available nutrients, stabilization of soil structure, and degradation of xenobiotic (foreign or strange) compounds. However, despite the fact that there is an exhaustive amount of literature available on the effects of manure on soil enzyme activities, there is no comprehensive overview of recent research findings that compares different management scenarios, manure types, and potentially new manure products or management. The purpose of this chapter is to provide a review of the response of enzyme activities to manure applications and their potential implications on soil biogeochemical cycling in agroecosystems. Additionally, this chapter intends to provide some perspective on specific areas where more information is warranted and pinpoint avenues for future research.

## 6.1 Introduction

Most (80–90 %) soil processes that are involved in the decomposition and transformation of nutrients from organic compounds occur through biochemical reactions mediated by enzymes. As summarized in Fig. 6.1, soil enzymes can be either intracellular (enzymes inside microbial cells) or extracellular (enzymes that have been released into soil solution and are attached to soil surfaces) (Kiss et al. 1975;

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Z. He and H. Zhang (eds.), *Applied Manure and Nutrient Chemistry for Sustainable Agriculture and Environment*, DOI 10.1007/978-94-017-8807-6_6,

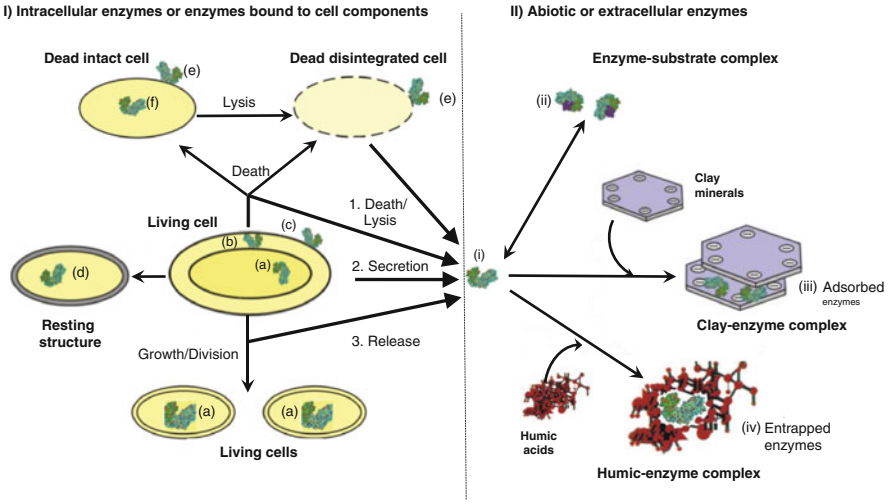


**Fig. 6.1** Soil enzymes are derived from two major sources: (**I**) Intracellular enzymes or enzymes bound to cell components which include (*a*) enzymes functioning within the cytoplasm of proliferating microbial, animal and plant cells, (*b*) enzymes restricted to the periplasmatic space of proliferating Gram-negative bacteria, (*c*) enzymes attached to the outer surface of viable cells with active sites extending into the soil environment, (*d*) enzymes within non-proliferating cells such as fungal spores, protozoa cysts, plant seeds and bacterial endospores, (*e*) enzymes attached to whole dead cells and cell debris, and (*f*) enzymes located inside intact dead cells; (**II**) Abiotic or extracellular enzymes, including (*i*) enzymes leaking from intact cells or released from dead or lysed cells that originated from the cell membrane or within the cell and which may survive for a short period in the soil solution, (*ii*) enzymes existing temporally as soluble or insoluble enzyme-substrate complexes, (*iii*) enzymes absorbed to the external or internal (i.e., within the lattices of 2:1 layer silicates) surfaces of clay minerals, or (*iv*) enzymes complexed with humic colloids via absorption, entrapment, or co-polymerization during humification. (This conceptual visual of the location of enzymes in soil was first developed by Klose 2003, and it is modified from Acosta-Martínez and Klose 2008)

Burns 1978; Ladd and Butler 1975; Nannipieri et al. 2002). The total enzyme activity of a soil (intracellular and extracellular) drives ecosystem services related to metabolic functioning, including: (1) decomposition of organic residues, (2) humification processes that stabilize soil organic matter (SOM), (3) the release of plant available nutrients, (4) the transformations of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) compounds via various processes, (5) stabilization of soil structure, and (6) degradation of xenobiotic (foreign or strange) compounds.

The application of manure to soil can restore ecosystem services related to nutrient cycling and SOM dynamics through the activities of enzymes involved in carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) cycling. However, the response of soil enzyme activities to anthropogenic disturbances, including manure application, is dependent upon inherent soil properties (e.g. pH, SOM and nutrient content, texture) and environmental factors (e.g. precipitation, temperature). The stabilization and protection of enzymes in soils occurs primarily via association with humic substances and clay complexes (Fig. 6.1), which can increase their

resistance to changes in environmental and climatic conditions that affect soil properties. In general, an enzyme-mediated reaction can increase about twofold for every 10 °C increase in temperature between 10 and 50 °C; however, very high temperatures can reduce soil enzyme activities due to inactivation (denaturation) at temperatures higher than 60–70 °C (Tabatabai 1994). In addition to temperature effects, each enzyme has a specific pH value at which the reaction rate is optimal, and at each side of this pH optimum the rate is lower. For example, phosphomonoesterases are classified as acid and alkaline phosphomonoesterases because there are two isoenzymes that contribute to the total activity with optimal activities in soils under acid and alkaline pH ranges, respectively. In short, the total enzyme activity of soil is very complex and depends on enzyme kinetics and stoichiometry of the different enzyme pools, as well as specific soil properties. Generally, enzyme activities are higher in clay and loam soils than in sandy soils following the clay and organic C contents (Acosta-Martínez and Klose 2008). Similarly, enzyme activities tend to decrease with increasing soil depth due to decreasing amounts of organic C and N along the soil profile (Senwo et al. 2007). Growing plants can also influence soil enzymes, and enzyme activities are often higher in the rhizosphere than in bulk soil (Waldrip et al. 2011).

Enzyme activities have been used as an early indicator of changes in soil quality, as they are more sensitive to changes in land use or management practices than other soil properties (Gregorich et al. 2006; Acosta-Martínez et al. 2007). One of the most common enzyme groups evaluated in soil are the dehydrogenases, which are oxidoreductases that are involved in the oxidation of multiple organic molecules with metabolic cofactors such as $NAD^+$ or $NADP^+$ as acceptor molecules (Dixon and Webb 1979). The study of dehydrogenase activity can provide information on overall organic matter oxidation through decomposition processes. Dehydrogenase activity is strictly intracellular, and thus reflects the total oxidative activities of the entire soil microbial community. Another commonly investigated group of enzymes are the hydrolases, which catalyze the hydrolysis of various chemical bonds (e.g., ester, glycosidic, ether and peptide bonds) by reaction with water. In soils, hydrolases play a key role in the cycling of C (e.g., β-glucosidase, α-galactosidase), C and N (e.g., β-glucosaminidase), N (e.g., urease, asparaginase, aspartase), P (e.g., alkaline phosphatase, acid phosphatase, phosphodiesterase), and S (e.g., arylsulfatase). While it has been noted that analysis of a number of enzymes involved in different reactions can provide an improved characterization of overall soil biogeochemical cycling, Dick (1994) emphasized the importance of carefully selecting enzymes that may reflect the influence of fertilization or other management practices on soil quality.

The application of manure and other organic amendments to soil has been suggested to exert a more important influence in maintaining soil microbial activity and diversity than other management practices, including conservation tillage (Dick et al. 1988). Most studies agree that significant increases in the activities of soil enzymes are observed in soils amended with animal manures (Khan 1970; Verstraete and Voets 1977; Dick et al. 1988; Martens et al. 1992) and green manures/crop residues (Verstraete and Voets 1977; Dick et al. 1988; Martens et al. 1992), as compared to unamended soils (Parham et al. 2002; Larkin et al. 2005;

Pérez-Piqueres et al. 2006). Some studies have reported a more pronounced increase in enzyme activities under manure applications than inorganic fertilizer (Bolton et al. 1985), while others have reported that repeated applications of inorganic fertilizers can suppress production of certain soil enzymes that are involved in cycling of a given nutrient (e.g. urease and amidase activities) compared to soils that receive manure additions (Dick et al. 1988; Dick 1992).

The response of soil enzyme activities may be short-lived with organic amendment applications, and many studies have reported that (Perucci 1990; Perucci and Giusquiani 1990; Martens et al. 1992). The effects of amendments on soil enzyme activities are difficult to predict because enzymes are substrate specific and the particular response of each enzyme to organic amendment may vary depending on the amendment type (e.g. swine lagoon slurry, solid beef cattle feedyard manure, poultry litter, sewage sludge, crop residues, etc.) and chemical composition (e.g. nutrient and OM content, pH, presence of inhibitory metals). Thus, manure types, application rates, timing and duration of application, application techniques (e.g. surface application of solid or liquid manure, fertigation, injection, incorporation via tillage), and management practices (e.g. tillage, no-till) may affect differently the response of enzyme activities, and this may vary for different soils and climatic and management conditions.

Generally, studies utilize a diverse group of enzymes involved in the mineralization of various N, P, C, and S compounds in order to evaluate the effects of manure on overall nutrient cycling and the different biochemical reactions that occur in soil. However, despite the fact that there is an exhaustive amount of literature on the effects of manure on soil enzyme activities, at present there is no comprehensive overview of recent research findings that compare different management scenarios on soil enzyme activities. The purpose of this chapter is to provide a review of the response of enzyme activities to manure applications and their implications on biogeochemical cycling in agroecosystems. In this chapter, we will provide an overview of the general response trends of various enzyme activities from studies that utilized different types of manure, rates of application, and application techniques (e.g. surface application or incorporation with tillage). Additionally, this chapter provides perspectives and suggestions for avenues of future research.

## 6.2 Mechanisms Involved in Changing Soil Enzyme Activities due to Manure Applications

There are two possible mechanisms for the effects of manure on enzyme activities of soils (Fig. 6.2). The first mechanism is via manure-induced changes to soil physicochemical properties, including bulk density, moisture content, pH, and soil temperature (Fig. 6.2a). This mechanism also includes the addition of nutrients

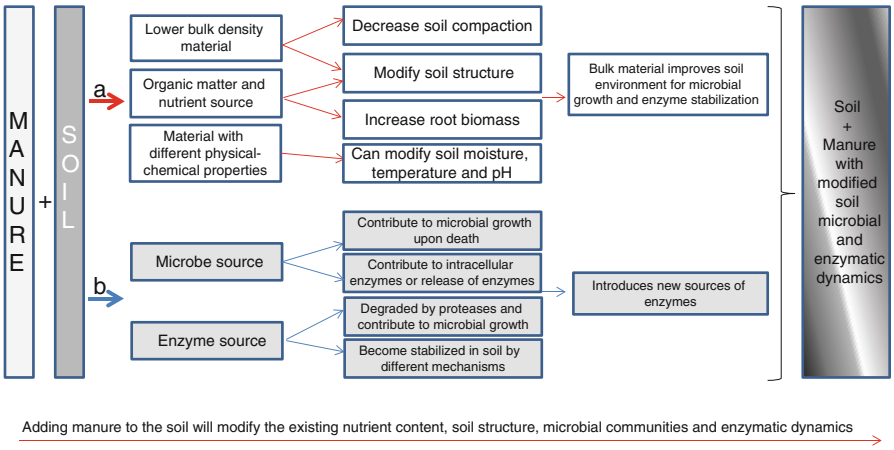


**Fig. 6.2** Two possible mechanisms for the effects of manure on enzyme activities of soils: (**a**) via manure-induced changes to soil physicochemical properties, including bulk density, moisture content, pH, and soil temperature; and (**b**) via microbial and enzyme load from the manure, which can contribute to microbial growth upon substrate release following death of manure-derived microbes, directly contribute microbes with intracellular enzymes and/or manure-derived extracellular enzymes

derived from manure, which can improve the soil conditions for microbial growth and increase enzyme stabilization (i.e., increase root biomass, decrease soil compaction). The second mechanism is related to the microbial and enzyme load from the manure, which can contribute to microbial growth upon substrate release following death of manure-derived microbes, directly contribute microbes with intracellular enzymes, and/or contribute manure-derived extracellular enzymes (Fig. 6.2b).

Although organic amendments often contain enzymes, studies have emphasized that the increase observed in enzyme activity in soils amended with organic residues is most likely due to stimulation of microbial activity by changes in soil properties (Fig. 6.2a), rather than the direct addition of amendment-derived enzymes or microbes (Fig. 6.2b) (Martens et al. 1992; Dick 1994). For example, Saison et al. (2006) explained that the effect of a compost amendment on soil enzyme activities was mainly due to the physicochemical characteristics of the compost matrix rather than compost-borne microorganisms. In addition, these researchers (Saison et al. 2006) saw no resilience of microbial characteristics during the study (6–12 months) after amendment with a high rate of compost. However, it has been noted that enzyme activities in manure can be comparable or higher than those found in soils. A recent study reported levels of dehydrogenase activity during initial stages of pig manure compositing are comparable to levels found in soils [160–250 mg triphenyl formazan (TPF) $kg^{-1}$ soil 24 $h^{-1}$] (Tiquia 2005). In addition, phosphatase activities in poultry manure were more than two orders of magnitude higher than commonly found in soils (Chap. 7, Sect. 7.4). Although studies consider that organic amendment-borne microorganisms may not have

long-term survival and therefore may have little effect on soil processes (Saison et al. 2006), the fact that a diverse pool of microbes are found in manure and other organic amendments (Durso et al. 2011) increases the possibility of some direct contribution of manure to soil enzyme pools. Thus, it is possible that among different organic amendments, the microbial and enzyme loads of manure can be significant enough to exert certain influence on the total enzyme activities, especially in low organic matter soils and/or sandy soils.

It is also important to recognize the effects of manure processing on the microbial and enzymatic load. For example, it has been observed that activities of phosphatases (acid and alkaline) and β-glucosidase in manure decreased sharply after just 3 days of active composting (Vuorinen 2000). This initial decrease was followed by an increase in these enzyme activities during the early curing phases, and then finally by an overall decrease in all activities. The study of Vuorinen (2000) also revealed that the bulking material used for composting affected the potential capacity and property for mineralization of P in the manure composts. It is clear that further research is warranted to comparatively evaluate the true contributions of different organic amendments, particularly manure, on the soil enzyme pools. In addition, more information is needed to quantify and qualify the extent of alteration and resilience of the inherent soil microbial community and enzymatic pool over time following manure application.

## 6.3 Assay Protocols and Sampling to Determine the Response of Enzyme Activities to Manure

### *6.3.1 Protocols for Assay of Enzyme Activities in Soil*

Most studies on the effects of manure on soil enzyme activities have predominantly used similar assay conditions (Nannipieri et al. 1978; Burns 1982; Sinsabaugh and Moorhead 1994; Tabatabai 1994) (Fig. 6.3, Table 6.1). Most assays for enzymes involved in C, P, and S cycling are based on determination of *p*-nitrophenol (PN) released during incubation of soil with buffered substrate solution (i.e., artificial substrate) under conditions determined to be optimal for the specific enzyme (Tabatabai 1994). The assays for enzymes involved in N cycling, such as the amidohydrolases (e.g. urease, aspartase, glutaminase, etc), are more commonly determined by back titration to quantify ammonium ($NH_4^+$) enzymatically released from specific amino acids or other substrates. Toluene has been commonly incorporated into enzyme assay solutions in order to inhibit microbial growth during the assay and stop further enzyme synthesis by living cells; however, some laboratories opt to omit toluene in order to reduce potential negative effects on the environment and human health following the handling and disposal of assay solutions (Acosta-Martínez and Tabatabai 2011). Among all enzyme assays, the assay for dehydrogenase activity, which is based on the colorimetric determination of the reduction of

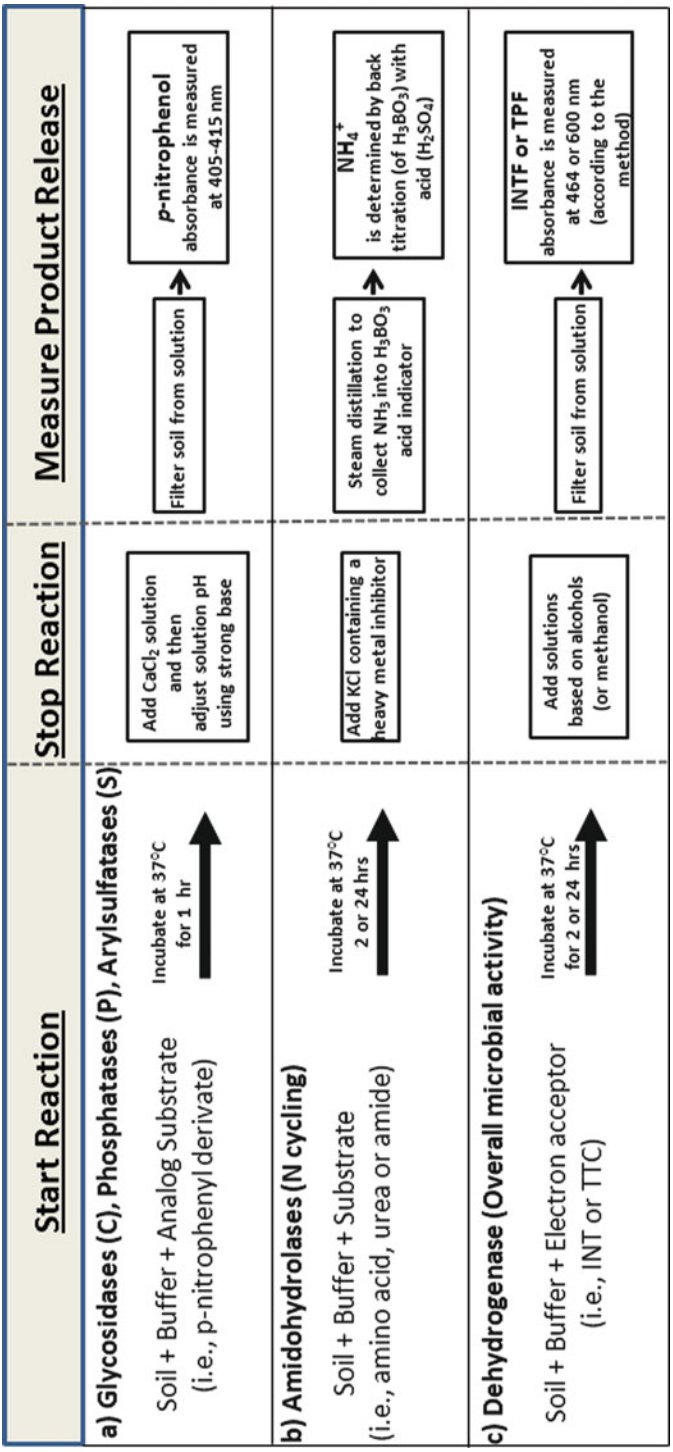


**Fig. 6.3** General steps and conditions for determining the most commonly studied soil enzyme activities with: (**a**) colorimetric determination of p-nitrophenol (PN) based assays (Tabatabai 1994); (**b**) distillation and titration of product for amidohydrolases (Tabatabai 1994), and (**c**) colorimetric determination of the reduction of different electron acceptor-indicators (Casida et al. 1964; Prosser et al. 2011). Additional information on enzyme activities and assays are found in Table 6.1

**Table 6.1** Description of assay conditions for most commonly assessed soil enzyme activities and their role in soil metabolic capacity and biogeochemical cycling (see assays described in Fig. 6.3)

| Recommended name and EC number | Role in soil metabolic function | Location in soils | Assay conditions | | | |
|---|---|---|---|---|---|---|
| | | | Substrate | Reaction | Optimum pH | See Fig. 6.3 |
| ***C cycling (Breakdown of C compounds)*** | | | | | | |
| β-Glucosidase (3.2.1.21) | Glycosidase. Cellulose degradation, produce glucose required as energy source for plants and microorganisms | Intracellular and extracellular | *p*-Nitrophenyl-β-D-glucopyranoside (10 mM) | Glucoside-R + $H_2O$ → Glucose + R-OH | 6 | a |
| Dehydrogenase (1.1.1.1) | Oxidoreductase. Catalyzes oxidation of various organic compounds during microbial respiration with the terminal acceptor being molecular oxygen | Strictly intracellular | 2, 3, 5-triphenyltetrazollium chloride (TTC) or 2-(4-iodophenyl)-3-(4-nitrophenyl) 5-phenyl-2H-tetrazolium chloride (INT) | Organic matter + TTC + $2e^-$ + $2H^+$ → oxidized organic matter + TPF | 7 | c |
| Both C and N cycling β-Glucosaminidase (3.2.1.30) | Chitin degradation, produce amino sugars which are a major form of mineralizable N in soil | Intracellular and extracellular | *p*-Nitrophenyl-N-acetyl-β-D glucosaminidine (10 mM) | R-N-acetyl-β-D glucosaminide → R-OH + N-acetyl-β-D-glucosamine | 5.5 | a |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***N cycling (Amidohydrolases: mineralization of organic N compounds to release plant available N, i.e., $NH_3$ or $NH_4^+$)*** | | | | | | |
| L-asparaginase (3.5.1.1) | Release of $NH_4^+$ from the amino acid asparagine | Intracellular and extracellular | Asparagine solution (0.5 M) | L-asparagine + $H_2O$ → L-aspartic acid + $NH_3$ | 10 | b |
| Amidase (3.5.1.4) | Catalyzes the hydrolysis of amides and produces $NH_3$ (C-N bonds other than peptide bonds in linear amides) | Intracellular and extracellular | Formamide solution (0.5 M) | $RCONH_2 + H_2O \rightarrow RCOOH + NH_3$ | 8.5 | b |
| Urease (3.5.1.5) | Catalyzes the hydrolysis of urea to $CO_2$ and $NH_3$ | Intracellular and extracellular | Urea solution (2 mg $ml^{-1}$) | $NH_2CONH_2 + H_2O \rightarrow CO_2 + 2NH_3$ | 10 | b |
| ***P cycling (Phosphatases: mineralization of organic P compounds into simpler inorganic P forms that can be taken up by plant roots, such as $H_2PO_4^-$ and $HPO_4^{2-}$)*** | | | | | | |
| Phosphodiesterase (3.1.4.1) | Produces phosphate monoesters | Intracellular and extracellular | *bis-p*-nitrophenyl phosphate (R-$Na_2$ 2$PO_4$) | R-$Na_2$ 2$PO_4$ + →R-$Na_2PO_4$ + R-OH | 8 | a |
| Acid Phosphatase (3.1.3.2) | Produces plant available phosphates and is predominant in acid soils | Intracellular and extracellular | *p*-Nitrophenyl phosphate (R-$Na_2PO_4$) | R-$Na_2PO_4 + H_2O \rightarrow$ R-OH + $Na_2HPO_4$ | 6.5 | a |
| Alkaline Phosphatase (3.1.3.1) | Produces plant available phosphates and is predominant in alkaline soils | More intracellular | *p*-Nitrophenyl phosphate (R-$Na_2PO_4$) | R-$Na_2PO_4 + H_2O \rightarrow$ R-OH + $Na_2HPO_4$ | 11 | a |
| ***S cycling (Sulfatases: mineralization of organic S compounds)*** | | | | | | |
| Arylsulfatase (3.1.6.1) | Produces plant available sulfates ($SO_4$) | Intracellular and extracellular | *p*-Nitrophenyl sulfate | R-$OSO_3^- + H_2O \rightarrow$ R-OH + $SO_4^{2-}$ | 5.8 | a |

2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) (Prosser et al. 2011) or 2,3,5-triphenyltetrazolium chloride (TTC) (Casida et al. 1964), can provide information of overall microbial activity in soil.

To date, it does not appear that more recent approaches, such as microplate-fluorimetric assays, have yet been utilized to evaluate the response of enzyme activities to manure. Microplate-fluorimetric assays are based on detection of 4-methylumbelliferyl (MUF) released by enzymatic hydrolysis of specific substrates when incubated with soil at the enzyme optimal pH. The differences of the detection of MUF via microplate-fluorimetric assays vs. PN with traditional assays have been discussed thoroughly by Deng et al. (2011, 2013), and the use of these new approaches can be considered in future research to investigate the response of enzyme activities to manure applications.

Interpretation of results from current enzyme assay protocols is limited by the lack of approaches available to distinguish among the location of enzymes in soil, as different pools contribute to total activity and these vary for different enzymes and soils (Nannipieri et al. 2002). For example, enzymes more significantly linked to intracellular origin/sources will be more closely related to actual microbial community composition (structure and diversity) of soil. This complication limits researchers' ability to identify the effect of manure on specific enzyme pools and determine how these pools contribute to specific soil processes and nutrient cycling following manure application.

An additional factor to be considered for evaluating soil management effects, such as manure applications, on enzyme activities as indicators of biogeochemical cycling is the fact that enzyme assays only provide information on potential activity, as optimum conditions are typically set in order to achieve maximum enzyme activity (buffer with optimum pH, saturated substrate solution, temperature, and synthetic substrates). These controlled assay conditions described are considered necessary to allow for comparisons across regions and climatic conditions; however, in reality, optimum conditions are rarely found in the field. Further studies are required in order to identify relationships between enzyme activities derived under optimal conditions and actual enzyme activities under variable field conditions.

### *6.3.2 Sampling Approaches to Evaluate Response of Enzyme Activities to Manure*

A chapter in an earlier published soil enzymology methodology book (Lorenz and Dick 2011) provides detailed procedural guidelines for soil sampling and pretreatment prior to enzyme analysis. Typically, enzyme activities are determined after air-drying soil subsamples. This use of air-dried soil is in contrast to microbiological assessments [e.g. DNA extraction, fatty acid methyl ester (FAME) analyses, chloroform fumigation methods for microbial biomass C or N], which

are generally determined on field-moist samples. It has been reported that air-drying has more of an effect on intracellular enzymes associated with the active microbial community than on extracellular enzymes stabilized in the soil matrix (Tabatabai 1994; Lorenz and Dick 2011). Some enzymes, including urease, β-glucosidase, cellulase, invertase, acid phosphatase and arylsulfatase, do not appear to be affected by air-drying (Acosta-Martínez et al. 2011; Lorenz and Dick 2011). Furthermore, analyzing enzyme activity in air-dried soils may better reflect the soil management history (Lorenz and Dick 2011), as enzymes can become stabilized in soil over years. The choice of how to store and keep soil samples prior to analyzing for enzyme activities is important in order to maintain consistency between samples and allow comparisons over time (i.e., long-term assessment). In the literature, most studies that evaluated the effects of manure applications on enzyme activities have used soils that were air-dried. With air-drying as a common factor, comparisons could be made between studies conducted on soils from different regions, where manures from different livestock types were applied, or where different manure application rates were used, and under different land use and management activities as shown in Table 6.2.

Among biochemical analyses, soil enzyme activities can be determined with simple analyses that require low labor costs compared with other methodologies (Ndiaye et al. 2000). However, analyses of multiple enzyme-mediated reactions that transform different nutrients are required to best evaluate changes that represent overall biochemical cycling. Thus, the selection of soil sampling times and number of enzyme activities to evaluate is a difficult decision, as it is necessary to balance between cost/time involved in sampling and laboratory analyses and the value of the index of biochemical cycling obtained (Dick et al. 1996). The frequency in which soil samples are taken depends on the overall goals of the specific study and it is important to initially decide if the study will focus on short- or long-term effects of manure application.

If short-term effects are being investigated, there should be frequent samplings after beginning manure applications to assist in identification of a status-quo; however, the number of sampling times can be reduced if long-term effects are being addressed (Schinner et al. 1996; Lorenz and Dick 2011). Samples can be taken more often during the first year(s) of manure applications (with samples taken at the same times every year to reduce the influence of seasonal variation) to address changes in enzyme activities which occur within the first years of manure applications. However, this sampling regime is insufficient to assess long-term trends for the soil scenario being evaluated. For longer-term field studies we recommend that soil sampling not be conducted within the first few months following manure application (Schinner et al. 1996), or after tillage or other management practices are performed, in order to avoid confounding effects. Samples in long-term studies should be taken at least two different times within the same year to determine trends that include seasonal changes. Alternatively, sampling at the same time for two (or more) consecutive years can provide an overview of the longer-term effects of manure applications on enzyme activities at that point in time and could reveal year to year climatic variations. As an example, Lorenz and

**Table 6.2** Literature review showing values of enzyme activities for manure-treated soils relative to untreated controls

| Enzyme activities[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| β-glucosidase | | Acid phosphatase | | Alkaline phosphatase | | Urease | | Dehydrogenase | |
| Control | Treated | Control | Treated | Control | Treated | Control | Treated | Control | Treated |
| 89 | 105 | | | 325 | 424 | | | | |
| 89 | 112 | | | 325 | 415 | | | | |
| 88 | 117 | 64 | 81 | 220 | 269 | | | | |
| 88 | 143 | 64 | 143 | 220 | 386 | | | | |
| | | | | | | 65 | 70 | 5.4 | 7.1 |
| | | | | | | 65 | 78 | 5.4 | 7.9 |
| | | | | | | 65 | 90 | 5.4 | 9.2 |
| 42 | 68 | | | | | 59 | 130 | | |
| 44 | 80 | 154 | 209 | 50 | 145 | 12 | 25 | | |
| | | 265 | 230 | 45 | 90 | | | 2.3 | 3.1 |
| 174 | 154 | | | 250 | 303 | 4.0 | 7.9 | 56 | 8.3 |
| | | 740 | 879 | | | 42 | 122 | | |
| | | | | 110 | 190 | 34 | 63 | 3 | 7.5 |
| 154 | 273 | 304 | 310 | nd | | | | | |
| | | 19 | 24 | 147 | 226 | | | 13.1 | 19.7 |
| | | | | 416 | 198 | 42 | 65 | 1.60 | 1.60 |
| | | | | 556 | 1,947 | | | 1.90 | 3.50 |
| | | | | 556 | 1,391 | | | 1.90 | 2.80 |
| | | | | 556 | 974 | | | 1.90 | 2.30 |
| 135 | 220 | | | 475 | 750 | | | | |
| 135 | 195 | | | 475 | 625 | | | | |
| 110 | 112 | | | 275 | 280 | | | | |
| 110 | 145 | | | 275 | 350 | | | | |
| 110 | 190 | | | 275 | 425 | | | | |
| 110 | 188 | | | 275 | 400 | | | | |
| 110 | 160 | | | 275 | 375 | | | | |
| 84 | 232 | 120 | 188 | 295 | 589 | 11 | 45 | 0.2 | 0.4 |
| 154 | 281 | 304 | 393 | | | | | | |
| | | 633 | 915 | 273 | 425 | 0.1 | 0.2 | 1.8 | 4.2 |
| | | | | | | 65 | 63 | 5.4 | 1.5 |
| | | | | | | 65 | 58 | 5.4 | 4.6 |
| | | | | | | 65 | 53 | 5.4 | 2.7 |
| 130 | 143 | 305 | 325 | 305 | 325 | 203 | 217 | 0.3 | 0.3 |
| 130 | 143 | 305 | 330 | 305 | 330 | 203 | 230 | 0.3 | 0.3 |
| | | | | 430 | 512 | 12.5 | 12.5 | | |
| 162 | 204 | 120 | 197 | 295 | 480 | 11 | 40 | 0.2 | 0.4 |

[a]Enzyme activities units vary: mg p-nitrophenol $g^{-1}$ soil $h^{-1}$ (for the first three enzymes), mg $NH_4$ $g^{-1}$ soil $h^{-1}$ (urease) and mg triphenyl formazan $g^{-1}$ soil $h^{-1}$ (for dehydrogenase)

[b]Manure properties are in Organic C/Total N/P unless preceded by OM (organic matter) or TC (total carbon); rates represent amendment amounts unless followed by N, which represents N application rate

[c]The section provides as much information possible given by each study/references. Goyal et al. (1993) is the only microcosms experiment, and Lalande et al. (2000) requires this clarification: *3.34 kg per $m^3$ total N, **90 $m^3$ per ha

| Manure information[b] | | | Study information[c] | | | |
|---|---|---|---|---|---|---|
| | | | | Soil pH | | |
| Type as named | Organic C/N/P (% DM) | Rate (Mg ha$^{-1}$ year$^{-1}$) | Soil type | Control (treated) | Length (years) | References |
| Beef | TC30.8/1.3/0.3 | 5.2 | Silt loam | 8.3 (8.3) | 3 | Acosta-Martínez et al. (2011) |
| Beef | TC30.8/1.3/0.3 | 10.3 | Silt loam | 8.3 (8.1) | 3 | Acosta-Martínez et al. (2011) |
| Beef | TC36.6/2.3/0.5 | 1.5 | Sandy loam | 7.9 (8.0) | 2 | Acosta-Martínez et al. (2011) |
| Beef | TC36.6/2.3/0.6 | 4.2 | Sandy loam | 7.9 (7.8) | 2 | Acosta-Martínez et al. (2011) |
| Beef | nd | 0.056 N | Clay loam | 7.5 (7.4) | 5 | Deng et al. (2006) |
| Beef | nd | 0.168 N | Clay loam | 7.5 (7.4) | 5 | Deng et al. (2006) |
| Beef | nd | 0.504 N | Clay loam | 7.5 (7.5) | 5 | Deng et al. (2006) |
| Beef+Straw | nd | 11.2 | Silt loam | 6.4 (7.0) | 64 | Bandick and Dick (1999) |
| Beef+Straw | nd | 22.4 | Silt loam | nd | 55 | Dick et al. (1988) |
| Cattle | nd | 0.067 N | Silt loam | 5.0 (5.3) | 100 | Parham et al. (2002) |
| Cattle | 11.4/1.1/0.7 | 75 | nd | 8.2 (8.0) | 29 | Liu et al. (2010) |
| Cow | nd | 5.2 | Clay loam | 5.5 (6.2) | 3 | Bhattacharyya et al. (2005) |
| Ovine | OM46/1.7/1.1 | 24 | Sandy-silty loam | 8.2 | 5 | Albiach et al. (2000) |
| Dairy | TC12.1/0.8/1.2 | 50.4 | Silty loam | 6.8 (6.8) | 3 | Acosta-Martínez et al. (2011) |
| Farmyard | 35/0.5/0.25 | * | Sandy loam | 8.3 | 34 | Mandal et al. (2007) |
| Farmyard | 32/1.6/0.8 | 5.6 | Sandy loam | 7.4 (7.3) | 11 | Goyal et al. (1999) |
| Farmyard | 28/1.1/nd | 90 | Sandy loam | 7.9 | 1 | Goyal et al. (1993) |
| Farmyard | 28/1.1/nd | 45 | Sandy loam | 7.9 | 1 | Goyal et al. (1993) |
| Farmyard | 28/1.1/nd | 15 | Sandy loam | 7.9 | 1 | Goyal et al. (1993) |
| Poultry | 29.2/4.0/3.3 | 6.7 | Clay | 8.4 (7.7) | 4 | Acosta-Martínez and Harmel (2006) |
| Poultry | 29.2/4.0/3.3 | 13.4 | Clay | 8.4 (7.8) | 4 | Acosta-Martínez and Harmel (2006) |
| Poultry | 29.2/4.0/3.3 | 4.5 | Clay | 7.9 (8.1) | 4 | Acosta-Martínez and Harmel (2006) |
| Poultry | 29.2/4.0/3.3 | 6.7 | Clay | 7.9 (7.9) | 4 | Acosta-Martínez and Harmel (2006) |
| Poultry | 29.2/4.0/3.3 | 9 | Clay | 7.9 (8.1) | 4 | Acosta-Martínez and Harmel (2006) |
| Poultry | 29.2/4.0/3.3 | 11.2 | Clay | 7.9 (7.8) | 4 | Acosta-Martínez and Harmel (2006) |
| Poultry | 29.2/4.0/3.3 | 13.4 | Clay | 7.9 (8.0) | 4 | Acosta-Martínez and Harmel (2006) |
| Poultry | nd | 33.3 | Sandy Clay loam | 7.9 | 3 | Martens et al. (1992) |
| Poultry | TC23.4/2.8/2.4 | 14.4 | Silt loam | 6.8 (6.5) | 3 | Acosta-Martínez et al. (2011) |
| Liquid Hog | * | ** | Silt loam | 6.5 | 18 | Lalande et al. (2000) |
| Swine effluent | nd | 0.056 N | Clay loam | 7.5 (7.4) | 5 | Deng et al. (2006) |
| Swine effluent | nd | 0.168 N | Clay loam | 7.5 (7.4) | 5 | Deng et al. (2006) |
| Swine effluent | nd | 0.504 N | Clay loam | 7.5 (7.2) | 5 | Deng et al. (2006) |
| Municipal solid | 2.8/2.1/nd | 12 | Clay loam | – | 6 | Crecchio et al. (2004) |
| Municipal solid | 2.8/2.1/nd | 24 | Clay loam | – | 6 | Crecchio et al. (2004) |
| Sewage sludge | nd | 7.6 | nd | – | 32 | Marschner and Marschner (2003) |
| Sewage sludge | nd | 33.3 | Sandy Clay loam | 7.9 | 3 | Martens et al. (1992) |

Dick (2011) suggested that mid-to-late spring or late fall might be optimal for sampling soil from cropping systems in temperate regions, as there will have been no recent fresh input of organic amendments or fertilizers. It is always important to avoid sampling after recent disturbances, such as tillage, as they can mask the effects of enzyme activities (Schinner et al. 1996; Lorenz and Dick 2011).

When the objective of a study is to evaluate the response of enzyme activities to different manure types and across different soils, it is important to use similar (or comparable) rates of applications and to measure soil enzyme activities at a time of the year when the climate is most stable and there have been no recent soil disturbances (e.g. tillage activities). Manure application rates can be based on target dry matter, N, or P additions. It is crucial to take into account the fact that manures can vary in moisture content, degree of decomposition, and concentrations of C and nutrients. Pagliari and Laboski (2012) analyzed physicochemical properties of 42 manure samples from seven livestock species that were collected from a variety of manure storages (lagoons, manure piles, bedded packs). These researchers found a wide range in concentrations of nutrients, C, moisture, and values for pH and EC, not only between the manures of different species, but also between manures from the same species. Thus, nutrient loads supplied to soils can differ between manures when applied based on specific nutrient requirements (e.g. N or P basis) or when applied on a mass basis (e.g. Mg $ha^{-1}$). These confounding effects can make it difficult to compare the effects different manure types have on enzyme activities. Manure properties can change soil conditions and influence environmental factors, particularly moisture and temperature, which affect soil chemical and biological properties (Aon and Colaneri 2001), especially under long-term manure applications. This is also very important when comparisons across soils and/or different manure types are intended. In addition to the factors already mentioned, other factors such as cropping history should be considered in order to produce a valid comparison of manure effects on soil enzyme activities. Lorenz and Dick (2011) suggest that for rhizosphere research it is important to identify key moments during the life cycle of the plant species of interest and sample accordingly. The same rationale holds true for research addressing the effects of manure, where care must be taken in sampling in order to reduce the number of factors that could confound analysis.

Lorenz and Dick (2011) also stressed the importance of characterizing a site before sampling in order to represent the horizontal and vertical spatial variability in soil physical and chemical properties. For a study on manure effects on soil properties, we also advise that the approach used for manure application (e.g., banding vs. broadcast) should be considered, as it is important in regard to appropriate depth for soil sampling and duration of the study (i.e., evaluation of long-term vs. short-term effects). We recommend reviewing literature on sampling approaches of soils under manure applications where manure is applied heterogeneously within the plot (e.g., liquid manure applied by banding) as compared to plots where manure solids or slurry are surface broadcast or applied by other methods that provide a more homogenous field coverage by manure (e.g., Tewolde et al. 2013). In order to fully evaluate the effects of manure application on enzyme activities, soil

samples should also be taken from control plots that have not received manure (i.e., plots that were unfertilized or received inorganic fertilizer), but that were managed similarly (e.g., tillage, crop rotations, land use) as manure-amended plots with the same soil type.

## 6.4 Comparing Enzyme Activities as Affected by Different Manures

### *6.4.1 The Role of the Manures Chemical Composition*

Among studies comparing the effects of manures and other organic amendments on enzyme activities, an important factor identified in enzyme response was the differences in amendment chemical composition in terms of the C:N ratios, quantity and quality of substrates, final products of reactions, cofactors, the presence of heavy metals or other inhibitory compounds, and other chemical characteristics (e.g., pH, etc). For example, a study with three different manure types (beef, poultry, and dairy) reported that beef manure applied to loamy soils promoted greater responses in activities of enzymes involved in C cycling (e.g., β-glucosidase, α-galactosidase) than did the other manure types (poultry and dairy) within the first 3 years of applications (Acosta-Martínez et al. 2011). This difference was likely due to the fact that the C content of beef manure was almost twice as high as the other manures. Additionally, when this study compared the enzyme activity response to poultry and dairy manure applications, acid phosphatase activity was greater in soil that received poultry manure than dairy manure. It is possible that this response could be explained by the lower pH of the poultry (pH $<$ 7) and dairy (pH $>$7) manures that were applied. This study (Acosta-Martínez et al. 2011) also explained that the low pH of the poultry manure caused a decrease in soil pH and subsequent increase in acid phosphatase activity. In general, acid phosphatase activity may respond (increase) independently of soil organic matter content when soil pH is decreased within a given range (Eivazi and Tabatabai 1977; Acosta-Martínez and Tabatabai 2001). In contrast, application of layer hen manure to an acidic (pH 5.0) soil from Maine increased soil pH in a study by Waldrip et al. (2011), but had little effect on either acid or alkaline phosphatase activities in soils. However, application of organic dairy manures to the same soil type resulted in increased acid phosphatase activity over that of soils that received inorganic fertilizer N, and this increase in phosphatase activity in manure amended soil was correlated to manure C:N ratios (Waldrip et al. 2012).

Substrate quality in manure and other organic amendments has been identified as an important determinant of how enzymes will respond independent of C or N content. For example, in a greenhouse study, soil amended with pea vine had greater protease and β-glucosidase activities than soil amended with beef manure when both were applied on an equivalent N basis (Fauci and Dick 1994). These

researchers (Fauci and Dick 1994) explained that the pea vine amendment contained less lignin than beef manure; therefore, the C compounds in pea vine were more readily metabolized by the microbial biota than beef manure and supported greater enzyme activities. Another study reported that an increase in β-glucosidase activity in soils amended with composted municipal solid wastes or uncomposted cow manure was not proportional to the quantity of C added with these two organic amendments (Marcote et al. 2001). The difference in response to these two amendments was likely due to organic matter quality or inhibitory effects of the high concentrations of heavy metals in municipal solid waste compost. These researchers (Marcote et al. 2001) also explained that the cellulose in the municipal solid waste (20 %) was derived primarily from paper and cardboard, while the cellulose in the manure (45 %) came mainly from more readily decomposable cereal straw that was used as animal bedding.

### *6.4.2 The Role of Soil Properties*

The response of soil enzymes to organic amendments may vary among different soils due to their inherent soil properties (e.g., soil pH, texture). To date, few studies have simultaneously evaluated the response of different soils to manure (i.e., similar sampling times, manure types and/or regions); therefore, little is known about how specific soil properties influence enzyme activities in regard to manure application. Soil texture can be an important characteristic influencing the response of enzyme activities to manure applications, and in a multi-location study (Acosta-Martínez et al. 2011) reported a faster response of enzyme activities within the first years following application of beef manure to a fine sandy loam in Colorado than to a silt loam in Kansas. In this study (Acosta-Martínez et al. 2011), it was proposed that differences in enzyme activity responses were likely due to lower SOM content and greater sand content in the Colorado sandy loam than the Kansas silt loam.

In two unrelated studies (Albiach et al. 2000; Bhattacharyya et al. 2005), different responses in enzyme activities were observed following application of manure or municipal solid wastes, which may be partially explained by differences in soil pH. For example, one study (Albiach et al. 2000) evaluated the response of several enzyme activities (urease, alkaline phosphatase, phosphodiesterase, arylsulfatase, and dehydrogenase) to equal rates of different organic amendments (municipal solid waste, sewage sludge, sheep manure, vermicompost, and a commercial humic acid solution) added to a horticultural soil. These researchers (Albiach et al. 2000) reported that after 4 years, the highest activities occurred following application of municipal solid waste, followed by sheep manure and sewage. Among the enzyme activities, arylsulfatase and alkaline phosphatase showed the greatest increase (threefold) in response to the addition of municipal solid waste. However, another study (Bhattacharyya et al. 2005) reported higher enzyme activities (urease and acid phosphatase) in soils that had received decomposed cow manure than in soils that received municipal solid waste. An important difference between these two

unrelated studies is that the study by Bhattacharyya et al. (2005), reporting lower activities under the municipal solid waste treatment, was conducted on an acidic soil (pH 5.5) in which the high metal concentration in the municipal solid waste may have become more accessible and inhibited enzyme activity or microbial growth. This same inhibitory effect was not observed with the municipal solid waste used in the study by Albiach et al. (2000), where enzyme activities were stimulated despite significantly higher concentrations of certain metals than found in sheep manure. In this study (Albiach et al. 2000), the soil pH was higher (pH 8.0), which would make metals more insoluble than in the lower pH soil of Bhattacharyya et al. (2005). Although there were other differences between these two studies that could have influenced the responses of enzyme activities to applied municipal solid wastes and manures (e.g., climate, soil genesis, texture, etc), it appears that soil pH was a major determinant controlling the response of enzyme activities to organic amendments.

## 6.5 Comparing the Response of Enzyme Activities to Various Rates of Manure

### *6.5.1 Studies Evaluating a Single Type of Manure Applied at Different Rates*

Important ecosystem implications related to both water and soil quality can be elucidated from studies evaluating the response of soil enzyme activities to differing manure application rates. Recent studies have discussed how the leaching potential of manure-N is partly regulated by the activity of enzymes of soil involved in N transformations (Deng et al. 2000; Schimel and Bennett 2004). Similarly, studies conducted in three states where different manures were applied for 4 years reported that higher poultry manure application rates (13.5 Mg $ha^{-1}$) to a silt loam caused an increase in acid phosphatase activity and did not result in levels of residual soil test P or Cu and Zn that were considered harmful to surface water or cropping systems (Sistani et al. 2010; Acosta-Martínez et al. 2011). As emphasized in the previous section, trends in enzyme activities following manure applications can be very dependent on soil type. For example, a study on a cultivated high clay soil reported that poultry litter applications of $\geq$6.7 Mg $ha^{-1}$ resulted in increased enzyme activities of C (β-glucosidase, α-galactosidase), C and N (β-glucosaminidase), P (alkaline phosphatase) and S (arylsulfatase) cycling after only four consecutive annual applications; however, these high rates also resulted in nutrient concentrations in excess of crop needs and created the potential for P loss in runoff (Harmel et al. 2004; Acosta-Martínez and Harmel 2006). These results were likely influenced by the impermeability (saturated hydraulic conductivity ~1.5 mm $h^{-1}$) and low infiltration capacity of the soil used in this study, which was a Texas Blackland Vertisol containing 55 % clay.

Evaluation of different rates of liquid pig amendments (liquid hog manure or pig slurry) on different soil types in two unrelated studies (Lalande et al. 2000; Plaza et al. 2004) revealed proportional increases in several enzyme activities up to a similar rate of organic amendment (90 $m^3$ $ha^{-1}$). Among the enzyme activities evaluated by Lalande et al. (2000), dehydrogenase, acid phosphatase and arylsulfatase showed the strongest response to liquid hog manure, while the lowest response was found for urease and alkaline phosphatase activities. However, both studies found a greater response of dehydrogenase activity to liquid pig manures than the other enzymes tested, and urease and phosphatase had the lowest levels of response. An important point raised by Lalande et al. (2000) about the response of enzyme activities to liquid manure applications is the possibility of anaerobic conditions created by higher rates of application (i.e., in their case 120 $m^3$ $ha^{-1}$). The points raised by Lalande et al. (2000) are very critical for soil quality and functioning because anaerobic conditions created by excess moisture from liquid manure could decrease microbial diversity and limit nutrient cycling and transformation over time in liquid manure treated soils.

### 6.5.2 *Comparison of Different Types of Manure Applied at the Same Rate*

The comparison of different manures applied at similar rates could allow better elucidation of the effects of substrate quality on enzyme activities. For example, Tejada et al. (2006) reported a proportional increase in enzyme activities when cotton gin compost or poultry manure were applied to soil at rates of 5, 8, and 10 Mg OM $ha^{-1}$; however, they also found higher activities (up to 30 %) of β-glucosidase, phosphatase, and arylsulfatase following application of poultry manure than cotton gin compost. Similarly, a study that compared the effects of swine effluent and beef manure applied to a semiarid soil at rates of 0, 56, 168 and 504 kg N $ha^{-1}$ (Deng et al. 2006) reported that swine effluent caused a decrease, or no change, in the enzyme activities regardless of application rate. In contrast, there was a proportional response of some enzyme activities (L-glutaminase, L-asparaginase, urease) to beef manure applied at the same rates. In the study by Deng et al. (2006), dehydrogenase activity was increased only by the highest application rate (504 kg N $ha^{-1}$) of beef manure and there was no change in β-glucosaminidase activity.

It seems that it is possible to identify certain types of manure that, depending upon nutrient distribution, have more influence on specific enzyme activities and greater impact on a given nutrient cycle. For example, several studies have demonstrated that there is a higher response of phosphatases to poultry manure or litter application regardless of the soil type, likely due to the high concentrations of P in poultry manure (Acosta-Martínez and Harmel 2006; Deng et al. 2006).

### 6.5.3 *Response of Enzyme Activities to Methods of Manure Application and Management Practices*

In addition to the effects of manure types and application rates on enzyme activities, manure application practices could also have an impact on enzyme activities. There are many methods of manure application and incorporation, including irrigation, broadcast spreading, band spreading, and injection into the soil. However, few studies have evaluated the effects of manure application method or incorporation on soil enzyme activities. Some factors that influence the response of microbial communities and enzyme activities under different incorporation techniques could be due to variation in manure moisture content and infiltration between surface application of slurries vs. broadcast spreading of solid manure, soil disturbance with injection or disk incorporation, and compaction from machinery.

Tillage alone can have significant effects on microbial communities and the activities of enzymes. According to phospholipid fatty acids (PFLA) indicators, it appears that no-tillage practices can cause shifts in microbial community structure towards higher fungal populations, as compared to soils under tillage, and this can lead to increases in enzyme activities in no-till plots (Roldán et al. 2005; Kennedy and Schillinger 2006). However, in a study where enzyme activities were evaluated following application of three manure types to loamy soils under no-till and conventional tillage practices, there was no difference between the two management practices for the first 2 years of manure application (Acosta-Martínez et al. 2011). It is clear that additional studies are required to better address the effect of tillage practices and manure management on soil enzyme activities. However, methods of manure application can differ depending on the manure, which makes it difficult to address this factor. For example, liquid manures (pig slurries, dairy lagoon waste) are generally injected or sprayed on the soil surface, whereas solid manures (poultry manure, beef feedyard manure) are more often applied by broadcast spreading and left on the soil surface or incorporated by disking or tillage. In addition, more recent technologies are emerging that can also inject solid manure into the soil. In general, the United States Environmental Protection Agency (USEPA) encourages manure incorporation following application (USEPA 2012) and many states prohibit surface application of manure on frozen soils, during a rainfall event, or when conditions are such that a runoff event could occur. Therefore, there may be limitations on the number of published studies available to evaluate how manure application practices influence enzyme activities.

## 6.6 Future Research Needs for Comprehensive Knowledge of the Effects of Manure on Enzyme Activities and Soil Nutrient Cycling

Management practices that minimize the addition of organic amendments to soils diminish the potential for increasing enzyme activity, which could affect the ability of soils to cycle and provide nutrients for plant growth (Dick et al. 1988). Manure can also provide more benefits to soil biogeochemical cycling than inorganic fertilizers or other management practices. However, it is not possible to completely generalize a specific response of enzyme activities to manure under diverse soil types, as different factors can influence their response (climatic conditions, rates of application, soil properties, etc.). As new challenges emerge, particularly due to climate change, the selection of manure management practices that lead to a long and sustainable enhancement of biogeochemical cycling and SOM dynamics will be crucial. Thus, to increase our understanding of biogeochemical cycling and SOM dynamics in agroecosystems that receive manure applications, it will be important to investigate enzyme activities across larger temporal and spatial scales in order to take into account the effects of differing management practices, climatic conditions, soil properties, and manure characteristics. Additionally, information of the changes in enzyme activities and other soil processes should be coupled to characterization of the composition of the microbial community in order to establish linkages between the microbial communities associated to shifts in enzyme activities involved in specific reactions and biogeochemical cycling.

In the U.S., there is a need for more comprehensive assessments on how regional management and manure application practices, such as manure application methods, tillage (conventional vs. no-till) and application rates will influence soil enzyme activities. Regional comparisons with the most common manure types will lead to better understanding of the changes in enzyme activities in specific soil types and under certain climatic conditions. However, establishing comparisons across regions will only be possible if enzyme assays are performed with similar protocols for the determination of enzyme activities. We also recommend that analyses be conducted on the activities of several enzymes involved in different reactions in order to maintain the ability to compare results within the literature and within the same location over time. This will allow for a more mechanistic understanding of how soil and environmental properties influence biochemical cycling and nutrient availability. Currently, there is little information on the effects of manure applications on many enzyme activities related to biogeochemical cycling (e.g., N cycling enzymes such as protease, β-glucosaminidase). The use of a diverse range of enzymes can also provide an indication of the substrate quality applied with various type of manure, as they are substrate-specific.

We found it difficult to obtain details on the soil and manure properties within the existing literature. This type of information is important to be incorporated into existing process-level models for simulating soil nutrient transformations and predicting plant nutrient uptake and crop yields [e.g., the Denitrification-Decomposition model, DNDC (Li et al. 1999)]. Through the use of modeling

approaches, there is potential for researchers to reach the next level of data interpretation and use, which is the integration of multiple enzyme activities and manure management scenarios into useful indexes for reaching a balance between desired improvements in soil biogeochemical cycling, crop yields, and environmental quality.

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