Chapter 2

Ecology of Extracellular Enzyme Activities and Organic Matter Degradation in Soil: A Complex Community-Driven Process

MATTHEW D. WALLENSTEIN, *Natural Resource Ecology Laboratory, Colorado State University, Colorado, USA*

RICHARD G BURNS, *University of Queensland, Brisbane, Australia*

Introduction

In fertile soils, microorganisms are surrounded by organic matter that is rich in carbon and the nutrients that are required for cell maintenance and growth. However, microbes cannot directly transport these macromolecules into the cytoplasm. Rather, they rely on the activities of the myriad of enzymes that they synthesize and release into their immediate environment. These extracellular enzymes depolymerize organic compounds and generate soluble, low-number oligomers and monomers that are then recognized by cell-wall receptors and transported across the outer membrane and into the cell.

Protein synthesis and enzyme production and secretion is energetically expensive, requires nitrogen (Schimel and Weintraub, 2003), and is ultimately debilitating unless there are equivalent nutritional rewards. Thus, the allocation of cell resources to enzyme synthesis and secretion must involve a dynamic balance between the investment of the precious resources allocated to the production of enzymes with the energy and nutrients gained as a result of their activity. However, soil is an inherently hostile environment for extracellular enzymes because once they leave the cell they are subject to denaturation, degradation, and inactivation through both biotic and abiotic mechanisms. At first glance, the microbial breakdown of organic macromolecules in soil looks to be an impossible task!

The locations and functions of enzymes in soil have been researched and discussed for decades (Burns, 1978, 1982; Burns and Dick, 2002; Nannipieri et al., 2002a; Caldwell, 2005; Wallenstein and Weintraub, 2008), have been the focus of three

recent International Conferences in Grenada, Spain; Prague, The Czech Republic) and, most recently, Viterbo, Italy and are now the focus of the Enzymes in the Environment Research Coordination Network (http://enzymes.nrel.colostate.edu). In the last decade, advances in molecular biology, microscopy, and analytical techniques plus some imaginative re-thinking (Allison, 2005; Bouws et al., 2008; Wallenstein and Weintraub, 2008) have begun to provide new insights into the ecology of extracellular enzymes. Another motivation for the many scientific advances in this subject is the need to understand the detail of how enzymes function in a large number of industrial, medical and environmental processes (Skujins, 1978). For example, those concerned with composting (Crecchio et al., 2004; Raut et al., 2008), waste water (Shackle et al., 2006) and sludge treatment (Alam et al., 2009) and the conversion of plant materials, including wood and straw residues, to fermentable sugars for bioethanol production (Wackett, 2008) are trying hard to understand the functions and how to improve the efficiency of the many enzymes involved. The paper and pulp industry invests resources into the study of extracellular enzyme producing microorganisms (Witayakran and Ragauskas, 2009) and as does the food industry which must control post-harvest spoilage and manage the polysaccharidic wastes arising from many processes (Bayer et al., 2007). The invasive, destructive and economically disastrous activities of phytopathogens (Kikot et al., 2009) and the complex enzymology of ruminant digestion (Morrison *et al.* 2009) also require a detailed knowledge of organic polymer solubilization and mineralization. Many potential organic pollutants are chemically complex and/or poorly soluble and these require extracellular catalysis (often by 'ligninases') prior to uptake, catabolism and detoxification (Nannipieri and Bollag, 1991). The rational and successful bioremediation of contaminated soil will depend on a thorough understanding of these enzymatic processes (Asgher et al., 2008; Wackett, 2009). Microbial enzymes to degrade adhesins, disrupt biofilms and repel colonizers are of interest to the antifouling industry (Kristensen et al., 2008) and are the subject of countless patents. The range of interests in extracellular enzymes seems endless.

Our well-founded concerns regarding the consequences of climate change on soil processes have stimulated a great deal of experimental research, modeling, and theorizing on soil organic matter formation and decomposition (Davidson et al., 2000; Kirschbaum,

2004; Eliasson et al., 2005; Fang et al., 2005; Jones et al., 2005; Knorr et al., 2005; Bradford et al., 2008). The recalcitrant polyphenolic and polysaccharidic fraction of soil is a long-term repository for sequestered carbon as well as being essential for crumb structure, soil stability, plant nutrient and water retention, microbial diversity and activities, and a host of properties that contribute to soil fertility and plant productivity. Increasing soil (and water) temperatures, elevated atmospheric carbon dioxide (Finzi et al., 2006), and more frequent wetting and drying cycles will change microbial community composition and accelerate growth and enzyme activities (Henry et al., 2005; Chung et al., 2007; Allison and Martiny, 2008) either directly or following their impact on plants. The consequences of these changes may include a decline in the humic component: the C sink becomes a flux (Melillo et al., 2002; Jones et al., 2003). Those attempting to assess the outcomes of global warming by generating predictive carbon cycle models (Luo, 2007) must take into account any predicted increases in soil enzyme activities and the associated decline in the hitherto recalcitrant humic matter (Davidson and Janssens, 2006).

The goal of this chapter is to describe the complexity and diversity of soil enzymes and the macromolecules that they degrade. We will also discuss some aspects of the regulation of extracellular enzyme synthesis and secretion and the many locations and multiple fates of these enzymes after they are released from the cytoplasm. The chemical, physical and biological properties of soil all affect enzyme diffusion, survival and substrate turnover, as well as the proportion of the product that is available to, and assimilated by, the producer cells. The ways in which microbes and their extracellular enzymes attempt to overcome the generally destructive or inhibitory properties of the soil matrix and the various strategies they adopt for effective substrate detection and utilization will be described.

Substrate and Enzyme Diversity in Soils

Extracellular enzymes in soils catalyze the degradation of organic matter primarily through hydrolytic and oxidative reactions. Hydrolytic enzymes are substrate-specific, in that their conformation enables them to catalyze reactions that cleave specific bonds (e.g., C-O and C-N bonds) that link monomers. On the other hand, oxidative

enzymes that act on broader classes of substrates that share similar bonds (e.g., C-C and C-O-C) use either oxygen (oxygenases) or hydrogen peroxide (peroxidases) as electron acceptors.

Although soil organic matter is traditionally classified into several broad groups based on solubility and molecular mass, its chemical structure varies widely at the molecular level (Piccolo, 2001; Kelleher and Simpson, 2006). Thus, an equally diverse suite of enzymes has evolved to access the carbon and nutrients contained, but not immediately bioavailable, in plant, animal and microbial detritus (Caldwell, 2005). Another factor contributing to the need for an enormous variety of soil extracellular enzymes is their interactions with the abiotic environment. For example, temperature is a strong controller of the three-dimensional physical structure of enzymes (conformation) as well as their configuration at surfaces such as clays (Daniel et al., 2008). The active site on an enzyme (both before and after complexing and sorption) may only be exposed and accessible to substrates under a narrow temperature range, resulting in thermal optima for the activity of each enzyme (Daniel et al., 2001) that is different from when the enzyme is in the solution phase. Similarly, pH can affect the conformation of enzymes and thus their activity (Niemi and Vepsalainen, 2005) and again especially at surfaces where hydrogen ion concentrations may be different to those in the bulk phase. Soil ion exchange capacity and charge density and distribution also will influence substrate-enzyme interactions and kinetics. Thus, the same or different microorganisms may, by neccessity, produce not only an arsenal of enzymes that target the same substrate but also multiple variants of individual enzymes.

For complex organic material, such as plant debris (composed of cellulose, hemicelluloses, pectins, starch and lignins), extracellular degradation requires the simultaneous and/or sequential activities of a large number of hydrolytic and oxidoreductive enzymes produced by a diverse community of bacteria and fungi. In the case of a plant leaf or a dead microbial cell (composed of peptidoglycans, lipoteichoic acids, lipopolysaccharides, glycoproteins, chitin, glucans and mannans), it is likely that more than 50 different extracellular enzymes are involved even before all the organic matter is transformed into the low-molecular-mass carbon and energy sources that can

enter the cell. Intuitively, the muti-enzyme processes necessary for successful biopolymer degradation in soils are improbable: cascades of enzymes secreted in an organized sequence and surviving long enough to function in concert to produce pentoses, hexoses, phenols, amino acids, amino sugars, etc. Despite this unlikely series of events, and the necessary contribution of diverse members of the decomposer community, the turnover of organic macromolecules is a constant and usually effective process. The complexity of soil organic matter degradation and the multiple roles of extracellular enzymes are well illustrated by the microbial ecology and enzymology of cellulose and lignin decay.

Cellulose, the most abundant form of fixed carbon, is a chemically simple yet structurally complex and insoluble polymer that is composed of linear chains of 5,000 or more glucose units held together with H-bonds to form rigid microfibrils. The microfibrils are then linked to hemicelluloses, pectins, glycoproteins and lignins. Different microbes have developed different and sometimes complex strategies to deal with cellulose in this natural state. But the rewards are great: an abundance of glucose. Basidiomycete and ascomycete fungi are major degraders of cellulose, typically employing a battery of extracellular hydrolytic enzymes (Baldrian and Valášková, 2008) including endo-1.4-β- glucanases or endocellulases (EC 3.2.1.4), cellobiohydrolases or exocellulases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21). Many fungi secrete all three of these enzymes although some bacteria retain β-glucosidases within the cytoplasm because they are able to transport cellobiose through the cell wall. In addition to the three principal cellulases, the overall oxidative decomposition of cellulose is a function of cellobiose dehydrogenase (EC1.1.99.18) and enzymically generated hydrogen peroxide and the resulting hydroxyl radicals. Other ill-defined extracellular enzymes (e.g. 'expansins', 'swollenins'), which help to loosen the structure of cell walls prior to cellulase penetration, have been recorded (Tsumuraya,1996; Kim et al., 2009). Some anaerobic bacteria use cellobiose phosphorylase (EC 2.4.1.20) to convert the dimer to glucose and glucose-1-phosphate.

The best known (and now sequenced) cellulose degrader, *Trichoderma reesei* (*Hypocrea jecorina*) has 30 or more glycosyl hydrolases, including seven endoglucanases, and a secretome containing greater than 100 proteins (Martinez et

al.,2008). Its close relative, *Trichoderma harzianum, produces* more than 250 extracellular proteins when grown on chitin or fungal cell walls (Suarez et al., 2005). Cellulolysis is less common in bacteria but there are many important strains, especially ruminant anaerobes and those found in high temperature environments. However, as with all microbes, growth conditions and substrates will strongly impact the synthesis and secretion of enzymes. The enzymology of cellulolysis and some of the strategies for degrading plant cell walls have been reviewed (Aro et al., 2005; Wilson, 2008) and a large number of celluloytic fungi and bacteria studied in detail.

Once in contact with their substrate, cellulases have a variety of ways in which they not only maintain their stability but also increase their activity. One mechanism relates to the all-important cellulose binding moieties (CBM) and their binding affinities (Hilden and Johansson, 2004)). Fungal and bacterial CBMs belong to many different families (Wilson, 2008) and serve to anchor the enzyme to its substrate at appropriate sites for the catalytic domain (CD) to cleave the β-1,4-linkages (Boraston et al., 2003). CBMs may also detach and slide across the fibrillar surface and thus move the associated CD along the cellulose chain. This relocates the CD and processively hydrolyzes the substrate (Jervis et al, 1997; Bu et al., 2009).

Not all cellulolytic microorganisms secrete the full complement of endo- and ecto-cellulases and they must, therefore, rely on other microbes in order to successfully degrade cellulose. This observation reinforces the notion of a community-driven process and it has been reported that endo- and ecto-cellulases from unrelated microorganisms can act synergistically sometimes with specific activites up to 15X those shown by the individual enzymes (Irwin et al., 1993). Logically, endocellulases should precede ectocellulases because they will expose more sites for attack.

The CAZy web site (http://www.cazy.org) currently lists 14 families containing cellulases. Why do fungi and bacteria express so many cellulases? There are many possible answers to this, as suggested previously, but certainly the physical diversity of the plant cell wall with its amorphous and crystalline cellulose regions, the many differing contributions of and associations with other structural polymers, and the variable chemical and physical properties of soil all play a part. The microbial

community needs cellulases for every occasion and in every situation in order to exploit the huge carbon and energy resource offered by plant residues.

Lignin, with which cellulose is usually associated, is a complex phenypropanoid and one of the major structural components of plant litter. In soil, the recalcitrant acid insoluble humic fraction is composed of lignin degradation products (vanillin, ferulic acid, guiacol, etc.) and many condensed polymers of these aromatics. Not surprisingly, lignin and its chemical cousin humic matter are amongst the most refractory components of soil organic matter.

Lignin degradation is carried out mainly by basidiomycetes (Hatakka, 1994; Osono, 2007), some ascomycetes, such as *Xylaria* spp. (Kellner et al., 2007) and certain actinobacteria (Kirby, 2006). Both saprotrophic (Valaskova et al., 2007) and ectomycorrhizal fungi (Chen et al., 2001) can produce lignin-degrading enzymes. Lignin is broken down by a suite of oxidative enzymes including laccases (E.C. 1.10.3.2), managanese peroxidases (EC 1.11.1.13), and lignin peroxidases (EC 1.11.1.14). The laccase gene, in particular, is widespread among bacteria (Alexandre and Zhulin, 2000; Claus, 2003), and the importance of bacteria in lignin degradation may be underestimated (Kellner et al., 2008). Lignin peroxidase (EC 1.11.1.14), cellobiose dehydrogenases (EC1.1.99.18) and pyranose-2-oxidases (EC 1.1.3.10) are also involved in lignin degradation (Baldrain et al., 2006; Nyanhongo et al., 2007). The well-known contribution of Fenton chemistry to the overall sequence (Rubilar et al., 2008) demands the input of enzymes generating hydrogen peroxide (e.g. glucose oxidase and glyoxal oxidase) as well as Fe^{2+} and Mn^{2+}. Clearly, as is the situation with cellulose, lignin degradation is a complex process involving a large number of enzymes (Wong, 2009).

sandenoj 6/18/10 11:39 AM
Comment: Rubilar et al. is not listed in the references

There are three classically described lignin degradation sequences involving white-rot, soft-rot, and brown-rot fungi (Osono, 2007). White-rot fungi are the only degraders able to completely mineralize lignin. They accomplish this through a combination of hydroxylation and demethylation, followed by oxidative degradation of the remaining aromatic rings by Mn-peroxidase. In contrast, brown-rot fungi modify lignin by removing methoxyl groups, but do not oxidase the aromatic rings. Soft-rot fungi such as ascomycetes and deuteromycetes, break down the middle lamella of the cell

wall, which acts to soften the lignin structure. Soft-rot fungi appear to produce peroxidases enzymes that are specific to lignin in hardwoods.

The white rot fungus *Phanerochaete chrysosporium* has more than 100 glycosyl hydrolases, in excess of 20 'ligninases' and a secretome of nearly 800 proteins (Vanden Wymelenberg et al., 2006) including lignin peroxidases (8), manganese peroxidases (8), other oxidoreductases (87), and glycoside hydrolases(90). The hydrogen peroxide required for peroxidase activity is produced by a suite of enzymes (glyoxal oxidases (EC1.1.3 -), superoxide dismutases (EC1.15.1.1), aryl alcohol oxidases (1.1.3.7). The lignin degrader *Coprinopsis cinerea* is predicted to secrete 1,769 proteins and, to date, 76 of these have been identified as enzymes (Bouws et al., 2008).

The physical structure of detritus is an important determinant of which compounds are enzymatically degraded first. Carbohydrates are often located on the outer fiber structures of litter, making them susceptible to the early colonizers of litter. On the other hand, some constituents that are otherwise relatively easily degraded can persist in litter until later stages because they are physically protected. Lignin acts as a barrier around cellulose and needs to be broken down to increase the access of cellulases to their substrate. Furthermore, lignin degradation *per se* is thought to be energetically unfavorable, thus, it is possible that brown-rot and soft-rot fungi degrade lignin only to access cellulose, other polymers or sources of nitrogen. Nonetheless, the relative amounts of acid insoluble substances tend to increase as lignocellulose decomposition occurs (Osono, 2007). It is probable that lignin degradation has to be coupled to celluloysis to generate an adequate carbon and energy supply and that fungi and bacteria act in concert to achieve this end.

Location and Stability of Extracellular Enzymes in Soil

Microbes and their extracellular enzymes must be capable of detecting, moving towards, and transforming organic debris to soluble monomers (or short oligomers) that are subsequently transported into the cytoplasm. As stated previously, the macromolecular components of living and dead plant, animal and microbial tissues are often physically and chemically associated with each other and sorb or entrap other low-

molecular-weight organic compounds. This presents a barrier that restricts microbial and extracellular enzyme access even to the otherwise vulnerable soluble constituents of organic matter. The substrates themselves may also be sequestered within soil components and this will reduce their accessibility to enzymes (Jastrow et al., 2007). Thus, degradation of macromolecules and more easily metabolised organics in soils requires not only enzyme production, but also physical contact of enzymes with their target substrates and the sites of catalysis. Furthermore, abiotic conditions must be within a range where enzymes can survive and activity can occur.

Extracellular enzymes may be associated with the microbial cell's plasma membrane, contained within and attached to the walls of the periplasmic space, cell wall and glycocalyx, or released into the soil aqueous phase (Figure 1; Sinsabaugh, 1994). The periplasm may provide Gram-negative bacteria with a reservoir of activity that is retained until an external signal for secretion is received – perhaps an efficient and rapid method for responding to the appearance of a potential substrate. Periplasmic enzymes may also process their proteins and enzymes en route to the extracellular environment in order to prepare them for the hostile 'real world.' For example, glycosylation may take place in the periplasm (Feldman et al., 2005). With regard to catalysis within the periplasm, the targets must be limited to low-molecular-mass structures that can be transported through the outer membrane. Thus, enzymes that degrade sugar dimers, such as cellobiose and mannose, and some phosphatases and nucleases are often (but not always) restricted to the periplasmic space and further transform their substrates immediately prior to transport into the cytoplasm.

sandenoj 6/18/10 11:39 AM
Comment: Is there supposed to be a Figure in this Chapter? The figure number does not appear to relate to the Sinsabaugh reference

Some extracellular enzymes are retained on the outer surface of the cell wall (i.e mural or peripheral enzymes) and are likely to be configured in such a way that their active sites are exposed and the zones that are most subject to attack by proteases are protected. Two other types of non-diffusing extracellular enzymes exist: those within the polysaccharidic coat that covers the cell or is part of a multicellular biofilm (Flemming and Wingender, 2002; Romani et al., 2008) and those organized within microscopically visible structures attached to, but protruding from, the cell wall. The latter, called cellulosomes, were first described for the anaerobic thermophile *Clostridium*

thermocellum, and have been much researched since (Bayer et al., 2004; Gold and Martin, 2007,;Bayer et al., 2008; Peer et al., 2009). *Clostridium thermocellum,* produces dozens of extracellular enzymes, including endoglucanases, exoglucanses, β-glucosidases, xylanases, lichenases, laminarinases, xylosidases, galactosidases, mannosidases, pectin lyases, pectin methylesterases, polygalacturonate hydrolases, cellobiose phosphorylases, and cellodextrin phophorylases (Lamed et al., 1983; Demain et al., 2005) and many of them may be contained within cellulosomes.

We now know that many anaerobic ruminant and some soil bacteria (including species belonging to the genera *Bacillus, Clostridium* and *Bacteriodes*), Archaea and fungi package their hydrolases at the cell surface. The enzymes are housed within somewhat rigid structures that vary from around 20nm to greater than 200nm in size and have a molecular mass in excess of one million. Cellulases (as well as hemicellulases, pectinases and xylanases) are arranged on a protein scaffold that provides an architecture that facilitates endo- and ecto- cleavage of polysaccharides. Cellulosomes may also contain proteases and secrete antibiotics (Schwarz and Zverlov, 2006) to give some the microbe some protection against competitors (see below). Based on our rapidly advancing knowledge of the bacterial proteome, the possibility of constructing cellulosome-like chimeras is under investigation (Mingardon et al., 2007). Some pathogenic bacteria actually bypass the risky secretion phase by translocating the extracellular enzymes directly into the target cell using secretory needle (Pastor et al., 2005). It is not know if such a mechanism functions in soil organic matter degradation.

Some enzymes that dissociate from the cell and are released into the aqueous phase will survive for a time. In fact, many extracellular enzymes are inherently more stable than their intracellular counterparts because they are glycosylated or have disulfide bonds. These modifications provide thermostability, a broad pH range for activity, and also some resistance to proteolysis. Other enzymes become stabilized through interactions with clay minerals and soil organic matter (Figure 1; (Burns, 1982; Nannipieri et al., 2002b) or tannins (Joanisse et al., 2007). In fact, much of the activity of some enzymes is associated with organic and inorganic colloids rather than being free in solution (Kandeler, 1990). Many of these bound enzymes are not extracellular *sensu*

sandenoj 6/18/10 11:39 AM
Comment: Where is this Figure?

stricta but rather they have become externalized as a result of the death and lysis of their parent cell. In fact, the same enzyme may be found in different locations. Regardless of their origins, stabilized enzymes often have reduced *in-situ* activity, as complexation can restrict substrate accessibility, occlude active sites, and cause conformational changes (Allison and Jastrow, 2006; Nannipieri, 2006; Quiquampoix and Burns, 2007). Enzymes stabilized as components of humic matter and associated with organomineral complexes may retain some activity (Tate, 2002). However, this will vary between specific enzymes. For example, (Gianfreda et al., 1995) found that when urease complexes with tannic acid are formed in the presence of ferric ions and aluminum hydroxide species, the enzyme retains its conformation and function. Pflug (1982) reported that clay-bound cellulase enzymes maintained activity, whereas starch degrading α-amylase and amyloglucosidase activities were completely inhibited following adsorption to clays. The same enzyme may even express different activities depending on its distribution between the various adsorptive soil fractions (Marx et al., 2005). Stabilization may also protect enzymes against proteases and other denaturing agents (Nannipieri et al., 1978; Nannipieri et al., 1988). It is possible that enzymatic function may be restored if enzymes are detached from organomineral complexes. However, irreversible deactivation is common with enzymes adsorbed to surfaces as a result of changes in protein conformation. If the enzyme is unfolded and the number of points of contact with the surface increase, more energy will be required to reverse the unfolding of the adsorbed enzyme, and may exceed the thermal energy available (Quiquampoix et al., 2002). Thus, the turnover time of extracellular enzymes complexed with humic molecules or adsorbed by clay minerals (or a combination of both) is likely to be longer than those free in the aqueous phase although their capacity to diffuse to distantly located substrates will be much reduced (Hope and Burns, 1985). However, turnover rates of enzymes in soils have not yet been measured—an important gap in our understanding of soil enzymology.

The active proportion of stabilized soil enzymes represents a reservoir of potential enzyme activity that may be important under some conditions. Indeed, it may represent the first catalytic response to changes in substrate availability in soils, and may serve as the originator of signaling molecules for the microbial community. Soil-bound enzymes

also may be a reservoir of potential activity in soils during periods when microbial biomass is low or shut down due to stressed conditions (Stursova and Sinsabaugh, 2008).

Identifying the functional locations of extracellular enzymes in soils and quantifying their individual contribution to the catalysis of a particular substrate are challenges yet to be overcome by soil biochemists (Wallenstein and Weintraub, 2008). The majority of research on soil enzymes has focused on quantifying potential enzyme degradation rates in soil slurries where substrate supply is non-limiting. In undisturbed soils, enzyme and substrate diffusion are restricted by the microscale spatial structure of the soil which limits water movement and isolates microsites. Several studies have examined the distribution of enzymes in relation to aggregate size (Henry et al., 2005; Dorodnikov et al., 2009) and Dong et al .(2007) used a novel approach to demonstrate that β-glucosidase activity was concentrated around plant roots. There has been some progress in imaging substrates and enzymes in soils since the early influential studies using scanning electron microscopy (Foster and Martin, 1981; Foster 1985) and confocal laser scanning microscopy (Alvarez et al., 2006) and atomic force microscopy show potential (Nigmatullin et al., 2004,\; Kaiser and Guggenberger, 2007). The use of autofluorescent proteins and enzymes warrants further investigation (Larrainzar et al., 2005). However, electron-dense soil minerals and humic substances and sequestration within clays and humates interfere with the visualization of enzymes. A potential advance that could overcome many of these limitations involves the use of Quantum Dots (Qdots), which are nano-scale crystals that emit in the near-infared wavelengths and are more photostable than current fluorophores (Michalet et al., 2005). Qdots that are quenched till they bind to their target enzyme (Blum et al., 2005; Blum et al., 2007) are already used widely in biomedical research to detect protease activity, and this technology could be adapted to soils (Whiteside et al., 2009). Direct measurement of *in-situ* enzyme activity would be the ultimate tool to fully understand the complex interactions between microbes, enzymes, and SOM.

Thermal Controls on Enzyme Activities

The conditions chosen to conduct enzyme assays in the laboratory have long been debated because the outcome will be determined by such as pH, substrate concentration,

water content and agitation. In addition to these methodological uncertainties, there are temporal and spatial variations ranging from the soil microenvironment to the geographic that render the interpretation of the data very difficult. A good example of the problem is the influence of temperature on enzyme activities (Wallenstein and Weintraub, 2008). In most ecosystems, soil temperatures vary on diel to seasonal time scales, and change in response to long-term climate trends. If we assume that over a certain range enzyme activity roughly doubles for every 10°C increase in temperature (i.e. $Q_{10} = 2$), then the effect of temperature may have a greater impact on in situ activity rates than seasonal fluctuations in enzyme potential at most sites. For example, (Wallenstein et al., 2009) developed a quantitative model of in-situ β-glucosidase activities based on seasonal lab-based measurements of potential activities at two temperatures, and using daily soil temperature data from an Arctic tundra site. They found that temperature explained 72% of the annual variation in predicted in-situ activities. Temperature had a larger influence on modeled enzyme activity than seasonal changes in enzyme pools. Clearly, temperature controls on enzyme activities needs to be further explored in other biomes.

The assumption that all enzymes are equally sensitive to temperature, or even that the same class of enzyme exhibits a consistent temperature sensitivity within a single site, has not been borne out in the literature. In fact, several studies have demonstrated that the temperature sensitivity of extracellular enzymes changes seasonally (Fenner et al., 2005; Koch et al., 2007a; Trasar-Cepeda et al., 2007; Wallenstein et al., 2009). The most likely explanation is that the measured enzyme pool consists of contributions from different enzymes and isoenzymes and these change with time, as do their microbial source(s) (Loveland et al., 1994; Sanchez-Perez et al., 2008). Consistent with this hypothesis, Di Nardo et al. (2004) found temporal changes in laccase and peroxidase isoenzymes during leaf litter decomposition. There is also some evidence for biogeographical patterns in enzyme temperature sensitivity. For example, many studies have observed that enzymes from microbes inhabiting cold environments have unusually low temperature optima (Huston et al., 2000; Coker et al., 2003; Feller and Gerday, 2003). Nonetheless, these observations suggest that microbes producing enzymes that maintain optimal activity under native soil conditions are favored. Thus, soil microbial

community composition is likely controlled to some extent through feedbacks with enzyme efficacy.

The accumulated evidence of numerous studies suggests a wide range in temperature sensitivities for different enzymes, and measured Q_{10} values are often much less than two (McClaugherty and Linkins, 1990; Frankenberger and Tabatabai, 1991a, b; Wirth and Wolf ,1992; Criquet et al., 1999; Parham and Deng, 2000; Elsgaard and Vinther, 2004). For example, Trasar-Cepeda et al. (2007) measured the Q_{10} of nine different enzymes in three different soils, and found that the Q_{10} at 20°C exceeded 2.0 only for a single enzyme (β-glucosidase) in one of the soils. Most of the enzymes had a Q_{10} closer to 1.5. The apparent temperature sensitivity of enzymes in lab assays with unlimited amounts of substrate and in without constraints to diffusion may differ markedly from in-situ temperature sensitivities. Even despite methodological concerns, there is insufficient data to assess the degree to which enzyme temperature sensitivity varies across spatial gradients or in response to other environmental factors. However, it is clear that temperature sensitivities differ within a single environment. For example, Koch et al. (2007b) found that at low temperatures, the relative temperature sensitivity of representative C-degrading enzymes was greater than aminopeptidases (which degrade N-rich proteins), suggesting that relative N availability could be decreased directly by temperature. Similarly, in the study by Wallenstein et al. (2009), N-degrading enzymes tended to have a lower Q_{10} (overall mean of 1.59) than C-degrading enzymes (overall mean of 2.07). If these enzymes are representative of the many enzymes involved in C and N cycling, we can conclude that without any changes in enzyme pools the relative *in situ* activity of these enzymes would change along with temperature, resulting in higher rates of C-mineralization relative to N-mineralization. Because different enzymes have different temperature sensitivities, changes in soil temperature may also alter the relative rates of decomposition for different components of soil organic matter. Therefore, seasonal changes in temperature can alter the balance of SOM components contributing to soil respiration without any changes in soil enzyme pools (or measured enzyme potentials). Natural or human-driven changes in climate could also alter the relative rate of decomposition of SOM components, and ultimately, the quantity and composition of SOM.

Well-established biochemical principles predict that the temperature sensitivity of enzyme-mediated reactions is directly related to the activation energy required to initiate that reaction (Davidson and Janssens, 2006). In other words, we would expect enzymatic reactions with higher activation energies to exhibit a greater degree of temperature sensitivity, all other factors being equal. If we extend this principle to organic matter decomposition, we would predict that the decomposition of low quality litter (i.e. litter with high molecular complexity), or specific steps in the decomposition sequence that have high activation energies, to be more temperature-sensitive than the decomposition of high quality (labile) litter or that involving enzymatic steps with low activation energies. This has been demonstrated under laboratory conditions by (Fierer et al., 2005) who found that the decomposition of less-labile litter is more temperature-sensitive than recalcitrant litter and that the temperature sensitivity of decomposition increases as decomposition progresses. Recent work suggests that this principle may also explain some of the observed variability in the temperature sensitivity of soil organic matter decomposition (Conant et al., 2008). However, it is important to note that organic carbon quality is likely to be only one factor regulating the apparent temperature sensitivity of decomposition in the field as litter carbon availability can be controlled by abiotic processes (soil aggregation, organo-mineral interactions) that may obscure the apparent carbon quality-temperature sensitivity interactions (von Lutzow and Kogel-Knabner, 2009). As temperatures increase, the proportion of assimilated substrate that is allocated to new biomass (substrate use efficiency) decreases (Steinweg et al., 2008). In other words, more of the substrate C is lost through respiration at higher temperatures. This has important implications for the long-term fate of detritus and could affect the proportion of detritus that is humified and stored in stable soil C pools versus that proportion returned to the atmosphere as CO_2, thus affecting the global C budget.

Regulation of Extracellular Enzyme Production

Our understanding of the microbial regulation of extracellular enzyme production in soil comes from either pure-culture studies, from patterns of enzyme potential across C and nutrient gradients or in response to experimental additions (Carreiro et al., 2000; Saiya-Cork et al., 2002; Michel and Matzner, 2003; Gallo et al., 2004; Allison and

Vitousek, 2005). In the case of pure culture studies, microbial physiology in artificial media does not reflect *in-situ* behavior, and few studies have represented the immense diversity of microbes present in soils. On the other hand, observations of variations in enzyme potentials in response to C or nutrient additions are suggestive of increased enzyme production, but are confounded with changes in enzyme stabilization, degradation, and changes in microbial biomass or community structure (Waldrop et al., 2000). Our understanding of the regulation of microbial enzyme production in soil is being advanced by studies using genomic, transcriptomic, and proteomic tools (Wallenstein and Weintraub, 2008).

In some cases, microbes may produce small amounts of extracellular enzymes regardless of substrate availability as a speculative sensing mechanism to detect substrate (Klonowska et al., 2002). When the substrate is present, these constitutive enzymes generate reaction products that induce additional enzyme synthesis. Once concentrations of products are sufficient to meet demand, enzyme production is down-regulated and returns to low constitutive levels (Chróst, 1991).

This is a process known as quorum sensing and has been well-described for many phytopathogens. For instance, *Erwinia caratovora* virulence factors are controlled by bacterial cell density and the local concentration of the self-produced signaling molecule acyl homoserine lactone (Barnard and Salmond, 2007). Gene products in this situation are pectin methyl esterase, pectic lyase and polygalacturonase that depolymerise the protective coat of the target seed or fruit and facilitate penetration and pathogenesis. Many other effector proteins pass into the host plant tissue and reduce its resistance to attack. Quorum sensing in the rhizosphere is believed to be an important controlling process for all sorts of catalytic activities (Pang et al., 2009).

Because microbial production of extracellular enzymes is carbon, nitrogen, and energy intensive, microbes should produce only enzymes such as polysaccharases when nutrients and soluble C are scarce (Koch, 1985), or to maintain the stoichiometry of microbial biomass (Cleveland and Liptzin, 2007). When a nutrient is available in the soil solution, microbes down-regulate or suppress their production of the enzymes that acquire that nutrient and thereby reduce the bioenergetic costs of manufacture (Pelletier

and Sygush, 1990; Chrost, 1991; Sinsabaugh and Moorhead, 1994). Polysaccharase production is increased when there is plentiful supply of soluble nitrogen (Sinsabaugh et al., 2002) while excess carbon may increase protease synthesis. Another expression of this control is that extracellular enzyme secretion is usually inversely related to specific growth rate. When particular nutrients are scarce, on the other hand, microbes secrete enzymes to liberate those nutrients from organic matter (Harder and Dijkhuizen, 1983). However, this strategy can only be successful if the appropriate organic substrates are present. As a result, the production of some extracellular enzymes may occur only in the presence of a suitable substrate or some other inducer (Allison and Vitousek, 2005). It is important to remember that the inducer molecule may not have to enter the cell to stimulate extracellular enzyme synthesis and release. Instead, it can bind to cell wall receptor proteins (sensory kinases) and initiate a sequence that passes the signal into the cell.

The successful depolymerization and subsequent metabolism of complex carbon and energy sources will be governed by the growth requirements of the attacking microorganisms and, in that context, C:N:P ratios (and yield coefficients) may be rate-limiting. Leaf litter generally has a C:N ratio in the range 20-80 and a C:P ratio of around 3000:1. Microorganisms, on the other hand, have C:N ratios of 5-10:1 and C:P ratios of 50-100:1 (Cleveland and Liptzin, 2007). Therefore, microbes degrading plant residues need not only the right compliment of penetrative enzymes but also ways to access additional N and P (and all the other elements that make up a microbial cell). One source will be dead microbial cells, although these are also composed of many complex polymers. A high proportion of nitrogen in soil will be in the form of proteinaceous compounds which will themselves require the activities of extracellular proteinases prior to microbial utilization (Geisseler and Horwath, 2008).

Because decomposition is a predominantly extracellular process that may take place remote from the cell, the diffusing enzymes can be destroyed or inactivated long before they locate a substrate. But even if the organic substrate is located and degraded, other microbes may intercept the products before the originating cell can benefit. These opportunistic ‘cheaters’ may not have invested any resources in extracellular enzyme

generation yet will reduce the efficiency of the entire process as far as the actual enzyme producer is concerned (Allison, 2005). However, some microbes employ antibiotics and enzymes to reduce this cheating or they rely on the activities of predators to control their rivals; in the rumen stomach protozoa may have this function (Modak et al., 2007). The various forms of mural enzymes overcome the 'highjacking' problem by delivering products at the cell surface. The coordination of enzyme production by quorum sensing and the spatial aggregation of enzyme producers also may mitigate or reduce competitive interference from cheater microbes (Ekschmitt et al., 2005). Of course, what might appear to us as 'selfish' may be part of a complex and poorly understood microbial community cooperation: the so-called cheaters might provide some direct or, more likely, indirect benefit to the cheated. Or, it may be that the benefits of a successful extracellular depolymerization far outweigh the disadvantages derived from some of the products being intercepted. Within a self-supporting microbial community, low levels of continuous enzyme secretion by a small proportion of the population might be effective because rapid up-regulation can occur once the substrate is detected and the cells receive the appropriate signal. But what proportion of the community can be sacrificed to ensure the success and stability of the remaining components? In this context it would be useful to produce an energy budget that compares expenditure to savings and interest within the microbial community and how this is modified subsequently in soil. Game theory analysis as it may apply to microbial strategies in soils warrants more attention (Velicer, 2003; Davidson and Surette, 2008) as does intraspecies variation and individual sacrifice for the benefit of the whole (Krug et al., 2008).

Conclusions

We have emphasized the role of microbial extracellular enzymes in the degradation of lignocelluloses, but equally detailed and sometimes speculative descriptions could be applied to chitin (Ekschmitt et al., 2005; Duo-Chuan, 2006), pectin (Abbott and Boraston, 2008), mannan (Dhawan and Kaur, 2007), and many other biopolymers. In addition, we have ignored a major component of the overall process of lignocellulolysis and carbon mineralization – that is the comminuting activities of invertebrates – especially ants (Ikeda-Ohtsubo and Brune, 2009), termites (Douglas,

2009) and earthworms (Nozaki et al., 2009) and the symbiotic activities of the cellulolytic microbes they cultivate (Silva et al., 2006) or harbor in their digestive tracts. The contribution that grazing ruminants and their gut microflora (Desvaux, 2005) is also part of the densely complex topic of macromolecule breakdown in soils.

An enhanced knowledge of extracellular enzyme function will have many practical applications including manipulating the soil for bioremediation, biocontrol, plant nutrient generation and availability, and C sequestration. There are also implications for plant pathology, biofuel production, and the impacts of climate change on soil enzyme activities. Protein survival and movement in soils and sediments is a broader issue and may have important human health consequences (Quiquampoix and Burns, 2007).

One of the greatest challenges in soil enzymology is to link the functional and ecological aspects of soil microbial extracellular enzyme activities to organic matter degradation. We are increasingly equipped with the analytical (electrophoretic, chromatographic, mass spectrometric – especially MALDI-TOF/MS), microscopical (fluorescence, scanning probe, atomic and ultrasonic force, confocal laser, scanning probe, differential interference , etc) , molecular (genomics, proteomics, metabolomics, secretomics, metagenomics (Metcalfe et al., 2002; Daniel, 2005; Fuka et al., 2008), biosensor (Gage et al., 2008) and bioinformatic tools to achieve this objective at both the individual genotype and community level. We must now begin to apply these tools to advance our understanding of the ecology and biochemistry of soil enzymology.

Are the activities of microbial enzymes in soil an example of organized chaos, ongoing selection processes or the outcome of an advanced and stable community organization? The answers to these fundamental questions and their application to the rational manipulation of soil should emerge within the next decade.

References

Abbott, D.W. and A.B. Boraston,. 2008. Structural biology of pectin degradation by *Enterobacteriaceae*. Microbiology and Molecular Biology Reviews 72:301-316.

Alam, M.Z., M.F. Mansor, and K.C.A. Jalal. 2009. Optimization of lignin peroxidase production and stability by Phanerochaete chrysosporium using sewage-treatment-plant sludge as substrate in a stirred-tank bioreactor. Journal of Industrial Microbiology and Biotechnology 36:757-764.

Alexandre, G., and I.B. Zhulin. 2000. Laccases are widespread in bacteria. Trends in Biotechnology, 18:41-42.

Allison, S.D. 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecology Letters 8:626-635.

Allison, S.D. and J.D. Jastrow. 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biology and Biochemistry 38: 3245-3256.

Allison, S.D. and J.B.H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences of the United States of America 105:11512-11519.

Allison, S.D. and P.M. Vitousek. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry 37:937-944.

Alvarez, V.M., I. von der Weid, L. Seldin, and A.L.S. Santos. 2006. Influence of growth conditions on the production of extracellular proteolytic enzymes in Paenibacillus peoriae NRRL BD-62 and Paenibacillus polymyxa SCE2. Letters in Applied Microbiology 43:625-630.

Aro, N., T. Pakula, and M. Penttila. 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. Fems Microbiology Reviews, 29:719-739.

Asgher, M., H.N. Bhatti, M. Ashraf, and R.L. Legge. 2008. Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation 19:771-783.

Baldrian, P., and V. Valášková, 2008. Degradation of cellulose by basidiomycetous fungi. FEMS Microbiology Reviews 32:501-521.

Baldrian, P. 2006. Fungal laccases - occurrence and properties. Fems Microbiology Reviews 30:215-242.

Barnard, A.M.L., and G.P.C. Salmond. 2007. Quorum sensing in Erwinia species. Analytical and Bioanalytical Chemistry 387,:415-423.

Bayer, E.A., J.P. Belaich, Y. Shoham, and R. Lamed., 2004. The cellulosomes: Multienzyme machines for degradation of plant cell wall polysaccharides. Annual Review of Microbiology 58:521-554.

Bayer, E A., R. Lamed, and M.E. Himmel. 2007. The potential of cellulases and cellulosomes for cellulosic waste management. Current Opinion in Biotechnology 18:237-245.

Bayer, E. A., R. Lamed, B.A.White, and H.J. Flint. 2008. From Cellulosomes to Cellulosomics. Chemical Record 8:364-377.

Blum, G., S.R. Mullins,K. Keren, M. Fonovic, C. Jedeszko, M.J. Rice, B.F. Sloane, and M. Bogyo. 2005. Dynamic imaging of protease activity with fluorescently quenched activity-based probes. Nature Chemical Biology 1:203-209.

Blum, G., G. von Degenfeld, M.J. Merchant, H.M. Blau, and M. Bogyo. 2007. Noninvasive optical imaging of cysteine protease activity using fluorescently quenched activity-based probes. Nature Chemical Biology 3,:68-677.

Boraston, A.B., E. Kwan, P. Chiu, R.A.J. Warren, and D.G. Kilburn. 2003. Recognition and hydrolysis of noncrystalline cellulose. Journal of Biological Chemistry 278: 6120-6127.

Bouws, H., A. Wattenberg, and H. Zorn. 2008. Fungal secretomes - nature's toolbox for white biotechnology. *A*pplied Microbiology and Biotechnology 80:381-388.

Bradford, M.A., C.A.Davies, S.D. Frey, T.R. Maddox, J.M. Melillo, J.E. Mohan, J.F. Reynolds, K.K. Treseder, and M.D. Wallenstein. 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters 11:1316-1327.

Bu, L.T., G.T. Beckham, M.F. Crowley, C.H. Chang, J.F. Matthews, YT.J. Bomble, W.S. Adney, M.E. Himmel, and M.R. Nimlos. 2009. The Energy Landscape for the Interaction of the Family 1 Carbohydrate-Binding Module and the Cellulose Surface is Altered by Hydrolyzed Glycosidic Bonds. J. Phys. Chem. B 113: 10994-11002.

Burns, R.G. 1978. Soil Enzymes. Academic Press, New York.

Burns, R.G. 1982. Enzyme-activity in soil - location and a possible role in microbial ecology. Soil Biology and Biochemistry 14:423-427.

Burns, R.G., and R.P. Dick. 2002. Enzymes in the environment: activity, ecology, and applications. Marcel Dekker, Inc, New York, New York, USA.

Caldwell, B.A. 2005. Enzyme activities as a component of soil biodiversity: A review. Pedobiologia 49:637.

sandenoj 6/18/10 11:39 AM
Comment: Should there be page numbers?

Matthew Wallenstein 6/20/10 8:49 PM
Deleted: 9999

Carreiro, M.M., R.L. Sinsabaugh, D.A. Repert, and D.F. Parkhurst, 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359-2365.

Chen, D.M., A.F.S. Taylor, R.M. Burke, and J.W.G. Cairney. 2001. Identification of genes for lignin peroxidases and manganese peroxidases in ectomycorrhizal fungi. New Phytologist 152:151-158.

Chróst, R.J. 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. p. 29-59 *In* R. J. Chróst (ed.) Microbial Enzymes in Aquatic Environments. Springer-Verlag, New York.

Chung, H.G., D.R. Zak, P.B. Reich, and D.S.Ellsworth, D. S. 2007. Plant species richness, elevated CO_2, and atmospheric nitrogen deposition alter soil microbial community composition and function. Global Change Biology 13:980-989.

Claus, H. 2003. Laccases and their occurrence in prokaryotes. Archives of Microbiology 179:145-150.

Cleveland, C.C., and D. Liptzin. 2007. C : N : P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85:235-252.

Coker, J.A., P.P. Sheridan, J. Loveland-Curtze, K.R.Gutshall, A.J. Auman, and J.E. Brenchley. 2003. Biochemical characterization of a beta-galactosidase with a low temperature optimum obtained from an Antarctic Arthrobacter isolate. Journal of Bacteriology 185:5473-5482.

Conant, R.T.,J.M. Steinweg, M.L. Haddix, E.A. Paul, A.F. Plante, and J. Six. 2008. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89:2384-2391.

Crecchio, C., M. Curci, M.D.R. Pizzigallo, P. Ricciuti, and P. Ruggiero. 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biology & Biochemistry 36: 1595-1605.

Criquet, S., S. Tagger, G. Vogt, G. Iacazio, and J. Le Petit, 1999. Laccase activity of forest litter. Soil Biology and Biochemistry 31:1239-1244.

Daniel, R., 2005. The metagenomics of soil. Nature Reviews Microbiology 3:470-478.

Daniel, R.M., M.J. Danson, and R. Eisenthal. 2001. The temperature optima of enzymes: a new perspective on an old phenomenon. Trends in Biochemical Sciences 26: 223-225.

Daniel, R.M., M.J. Danson, R. Eisenthal, C.K. Lee, and M.E. Peterson. 2008. The effect of temperature on enzyme activity: new insights and their implications. Extremophiles 12:51-59.

Davidson, C.J., and M.G. Surette. 2008. Individuality in Bacteria. Annual Review of Genetics 42:253-268.

Davidson, E.A. and I.A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature: 440:165.

Davidson, E.A., S.E. Trumbore, and R. Amundson. 2000. Biogeochemistry - Soil warming and organic carbon content. Nature 408:789-790.

Demain, A.L., M. Newcomb, and J.H.D. Wu,. 2005. Cellulase, Clostridia, and Ethanol. Microbiol. Mol. Biol. Rev 69:124-154.

Desvaux, M. 2005. The cellulosome of Clostridium cellulolyticum. Enzyme and Microbial Technology 37:373-385.

sandenoj 6/18/10 11:39 AM
Comment: Is this a journal? If so , it should be names and the issue number included. A book? Publisher information

Dhawan, S., and J. Kaur. 2007. Microbial mannanases: An overview of production and applications. Critical Reviews in Biotechnology 27:197-216.

Di Nardo, C., A. Cinquegrana, S. Papa, A. Fuggi, and A. Fioretto. 2004. Laccase and peroxidase isoenzymes during leaf litter decomposition of Quercus ilex in a Mediterranean ecosystem. Soil Biology and Biochemistry 36:1539-1544.

Dong, S., D. Brooks, M.D. Jones, and S.J. Grayston. 2007. A method for linking in situ activities of hydrolytic enzymes to associated organisms in forest soils. Soil Biology and Biochemistry 39:2414-2419.

Dorodnikov, M., E. Blagodatskaya, S. Blagodatsky, S. Marhan, A. Fangmeier, and Y. Kuzyakov. 2009. Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. Global Change Biology 15:1603-1614.

Douglas, A.E. 2009. The microbial dimension in insect nutritional ecology. Functional Ecology 23:38-47.

Duo-Chuan, L. 2006. Review of fungal chitinases. Mycopathologia 161:345-360.

Ekschmitt, K., M.Q. Liu, S. Vetter, O. Fox, and V. Wolters. 2005. Strategies used by soil biota to overcome soil organic matter stability - why is dead organic matter left over in the soil? Geoderma 128:167-176.

Eliasson, P.E., R.E. McMurtrie, D.A. Pepper, M. Stromgren, S. Linder, S., and G. I. Agren. 2005. The response of heterotrophic CO_2 flux to soil warming. Global Change Biology 11:167-181.

Elsgaard, L., and F.R. Vinther,. 2004. Modeling of the fine-scale temperature response of arylsulfatase activity in soil. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 167:196-201.

Fang, C.M., P. Smith, J.B. Moncrieff, and J.U. Smith. 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature: 433:57-59.

Feldman, M.F., M. Wacker, M. Hernandez, P.G. Hitchen, C.L. Marolda, M. Kowarik, H.R. Morris, A. Dell, M.A. Valvano, and M. Aebi. 2005. Engineering N-linked protein glycosylation with diverse O antigen lipopolysaccharide structures in Escherichia coli. Proceedings of The National Academy of Sciences of The United States of America 102:3016-3021.

Feller, G., and C. Gerday. 2003. Psychrophilic enzymes: Hot topics in cold adaptation. Nature Reviews Microbiology 1:200-208.

Fenner, N., C. Freeman, and B. Reynolds. 2005. Observations of a seasonally shifting thermal optimum in peatland carbon-cycling processes; implications for the global carbon cycle and soil enzyme methodologies. Soil Biology and Biochemistry 37:1814.

Fierer, N., J.M. Craine, K. McLauchlan, and J.P. Schimel., 2005. Litter quality and the temperature sensitivity of decomposition. Ecology 86:320-326.

Finzi, A .C., R.L. Sinsabaugh, T.M. Long, and M.P. Osgood. 2006. Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. Ecosystems 9:215-226.

Flemming, H.C., and J. Wingender. 2002. What biofilms contain - Proteins, polysaccharides, etc. Chemie in Unserer Zeit 36:30-42.

Foster, R., 1985. In situ localization of organic matter in soils. Questiones Entomologicae 21:609-633.

Foster, R., and J.K. Martin. 1981. In situ analysis of soil components of biological origin. p. 75-110 *In* E.A. Paul and J.N. Ladd, (ed.) Soil Biochemistry. Marcel Dekker, New York.

Frankenberger, W.T., and M.A. Tabatabai. 1991a. L-Asparaginase Activity Of Soils. Biology and Fertility of Soils 11:6-12.

Frankenberger, W.T., and M.A. Tabatabai. 1991b. L-Glutaminase Activity Of Soils. Soil Biology and Biochemistry 23:869-874.

Fuka, M.M., M. Engel, A. Gattinger, U. Bausenwein, M. Sommer, J.C. Munch, and M. Schloter. 2008. Factors influencing variability of proteolytic genes and activities in arable soils. Soil Biology and Biochemistry 40:1646-1653.

Gage, D.J., P.M. Herron, C.A. Pinedo, and Z.G. Cardon. 2008. Live reports from the soil grain - the promise and challenge of microbiosensors. Functional Ecology 22: 983-989.

Gallo, M., R. Amonette, C. Lauber, R.L. Sinsabaugh, and D.R. Zak,. 2004. Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. Microbial Ecology 48:218-229.

Geisseler, D., and W.R. Horwath. 2008. Regulation of extracellular protease activity in soil in response to different sources and concentrations of nitrogen and carbon. Soil Biology and Biochemistry 40:3040-3048.

Gianfreda, L., M.A. Rao, and A. Violante. 1995. Formation and activity of urease-tannate complexes affected by aluminum, iron, and manganese. Soil Science Society of America Journal 59: 805-810.

Gold, N. D., and V.J.J. Martin. 2007. Global view of the Clostridium thermocellum cellulosome revealed by quantitative proteomic analysis. Journal of Bacteriology 189:6787-6795.

Harder, W., and L. Dijkhuizen. 1983. Physiological responses to nutrient limitation. Annual Review of Microbiology 37:1-23.

Hatakka, A. 1994. Lignin-modifying enzymes from selected white-rot fungi - production and role in lignin degradation. Fems Microbiology Reviews 13:125-135.

Henry, H.A.L., J.D. Juarez, C.B. Field, and P.M. Vitousek. 2005. Interactive effects of elevated CO_2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. Global Change Biology 11:1808-1815.

Hilden, L., and G. Johansson. 2004. Recent developments on cellulases and carbohydrate-binding modules with cellulose affinity. Biotechnology Letters 26: 1683-1693.

Hope, C.F.A., and R.G. Burns. 1985. The barrier-ring plate technique for studying extracellular enzyme diffusion and microbial-growth in model soil environments. Journal of General Microbiology 131:1237-1243.

Huston, A. L., B.B. Krieger-Brockett, and J.W. Deming. 2000. Remarkably low temperature optima for extracellular enzyme activity from Arctic bacteria and sea ice. Environmental Microbiology 2:383-388.

Ikeda-Ohtsubo, W., and A. Brune. 2009. Cospeciation of termite gut flagellates and their bacterial endosymbionts: Trichonympha species and 'Candidatus Endomicrobium trichonymphae'. Molecular Ecology 18:332-342.

Irwin, D.C., M. Spezio, L.P. Walker, and D.B. Wilson. 1993. Activity studies of 8 purified cellulases - specificity, synergism, and binding domain effects. Biotechnology and Bioengineering 42:1002-1013.

Jastrow, J.D., J.E.Amonette, and V.L. Bailey. 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Climatic Change 80:5-23.

Jervis, E.J., C.A. Haynes, and D.G. Kilburn. 1997. Surface diffusion of cellulases and their isolated binding domains on cellulose. Journal of Biological Chemistry 272: 24016-24023.

Joanisse, G.D., R.L. Bradley, C.M. Preston, and A.D. Munson. 2007. Soil enzyme inhibition by condensed litter tannins may drive ecosystem structure and processes: the case of Kalmia angustifolia. New Phytologist 175:535-546.

Jones, C.D., P. Cox, and C. Huntingford. 2003. Uncertainty in climate-carbon-cycle projections associated with the sensitivity of soil respiration to temperature. Tellus Series B-Chemical and Physical Meteorology 55:642-648.

Jones, C., C. McConnell, K. Coleman, P. Cox, P. Falloon, D. Jenkinson, and D. Powlson 2005. Global climate change and soil carbon stocks; predictions from two contrasting models for the turnover of organic carbon in soil. Global Change Biology 11:154-166.

Kaiser, K., and G. Guggenberger. 2007. Sorptive stabilization of organic matter by microporous goethite: sorption into small pores vs. surface complexation. European Journal of Soil Science 58:45-59.

Kandeler, E., (1990). Characterization of free and adsorbed phosphatases in soils. Biology and Fertility of Soils 9:199-202.

Kelleher, B.P., and A.J. Simpson. 2006. Humic Substances in Soils: Are They Really Chemically Distinct? Environmental Science and Technology 40:4605-4611.

Kellner, H., P. Luis, and F. Buscot, 2007. Diversity of laccase-like multicopper oxidase genes in Morchellaceae: identification of genes potentially involved in extracellular activities related to plant litter decay. FEMS Microbiology Ecology, 61:153-163.

Kellner, H., P. Luis, B. Zimdars, B. Kiesel, and F. Buscot. 2008. Diversity of bacterial laccase-like multicopper oxidase genes in forest and grassland Cambisol soil samples. Soil Biology and Biochemistry 40:638-648.

Kikot, G.E., R.A. Hours, and T.M. Alconada. 2009. Contribution of cell wall degrading enzymes to pathogenesis of Fusarium graminearum: a review. Journal of Basic Microbiology 49:231-241.

Kim, E.S., H.J. Lee, W.G. Bang, I.G. Choi, and K.H. Kim. 2009. Functional Characterization of a Bacterial Expansin From Bacillus subtilis for Enhanced Enzymatic Hydrolysis of Cellulose. Biotechnology and Bioengineering 102: 1342-1353.

Kirby, R. 2006. Actinomycetes and lignin degradation. Advances in Applied Microbiology 58:125-168.

sandenoj 6/18/10 11:39 AM
Comment: Is this an error – what should it be?

Matthew Wallenstein 6/20/10 8:50 PM
Deleted:

sandenoj 6/18/10 11:39 AM
Comment: Is the + acceptable?

Matthew Wallenstein 6/20/10 8:52 PM
Deleted: Pages 125-+

Matthew Wallenstein 6/20/10 8:52 PM
Deleted: ,

Matthew Wallenstein 6/20/10 8:52 PM
Deleted: Vol

Kirschbaum, M.U.F. 2004. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? Global Change Biology 10: 1870-1877.

Klonowska, A., C.Gaudin, A. Fournel, M. Asso, J. Le Petit, M. Giorgi, and T. Tron. 2002. Characterization of a low redox potential laccase from the basidiomycete C30. European Journal of Biochemistry 269:6119-6125.

Knorr, W., I.C. Prentice, J.I. House, and E.A. Holland. 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433:298.

Koch, A.L. 1985. The macroeconomics of bacterial growth. p. 1-42 *In* M. Fletcher and G.D. Floodgate (ed.) Bacteria in their Natural Environments. Academic Press, London.

Koch, O., D. Tscherko, and E. Kandeler. 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. Global Biogeochem. Cycles, doi:10.1029/2007GB002983.

Kristensen, J.B., R.L. Meyer, B.S. Laursen, S. Shipovskov,F. Besenbacher, and C.H. Poulsen. 2008. Antifouling enzymes and the biochemistry of marine settlement. Biotechnology Advances 26:471-481.

Krug, D., G. Zurek, O. Revermann, M. Vos, G.J. Velicer, and R. Muller. 2008. Discovering the hidden secondary metabolome of Myxococcus xanthus: a study of intraspecific diversity. Applied and Environmental Microbiology 74:3058-3068.

Matthew Wallenstein 6/20/10 8:53 PM
Deleted: a

sandenoj 6/18/10 11:39 AM
Comment: This appears to be the same as the previous reference. Do you want to eliminate one of them and therefore remove the a and b? Also, there is a volume number but no page numbers in 2007b.

Matthew Wallenstein 6/20/10 8:52 PM
Deleted: Koch, O., D. Tscherko, and E. Kandeler. (2007b). Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. *s*Global Biogeochemical Cycles, 21.

Matthew Wallenstein 6/20/10 8:53 PM
Formatted: Indent: Left: 0", First line: 0"

Lamed, R., E. Setter, and E.A. Bayer. 1983. Characterization of a cellulose-binding, cellulase-containing complex in Clostridium thermocellum. J. Bacteriol.156:828-836.

Larrainzar, E., F. O'Gara, and J.P. Morrissey. 2005. Applications of autofluorescent proteins for in situ studies in microbial ecology. Annual Review of Microbiology 59:257-277.

Loveland, J., K. Gutshall, J. Kasmir, P. Prema, and J.E. Brenchley. 1994. Characterization of Psychrotrophic Microorganisms Producing Beta-Galactosidase Activities. Applied and Environmental Microbiology 60:12-18.

Luo, Y. Q. 2007. Terrestrial carbon-cycle feedback to climate warming. Annual Review of Ecology Evolution and Systematics 38:683-712.

Martinez, D., R.M. Berka, B. Henrissat, M. Saloheimo, M. Arvas, S.E. Baker, J. Chapman, O. Chertkov, P.M. Coutinho, D. Cullen, E.G.J. Danchin, I.V. Grigoriev, P. Harris, M. Jackson, C.P. Kubicek, C.S. Han, I. Ho, L.F. Larrondo, A.L. de Leon, J.K. Magnuson, S. Merino, M. Misra, B. Nelson, N. Putnam, B. Robbertse, A.A. Salamov, M. Schmoll, A. Terry, N. Thayer, A. Westerholm-Parvinen, C.L. Schoch, J. Yao, R. Barbote, M.A. Nelson, C. Detter, D. Bruce, C.R. Kuske, G. Xie, P. Richardson, D.S. Rokhsar, S.M. Lucas, E.M. Rubin, N. Dunn-Coleman, M. Ward, and T.S. Brettin. 2008. Genome sequencing and analysis of the biomass-degrading fungus Trichoderma reesei (syn. Hypocrea jecorina). Nature Biotechnology 26:553-560.

Marx, M.C., E. Kandeler, M. Wood, N. Wermbter, and S.C. Jarvis. 2005. Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biology and Biochemistry, 37:35-48.

McClaugherty, C.A., and A.E. Linkins. 1990. Temperature Responses Of Enzymes In Two Forest Soils. *S*oil Biology and Biochemistry 22:29-33.

Melillo, J.M., P.A. Steudler, J.D. Aber, K. Newkirk, H. Lux, F.P. Bowles, C. Catricala, A. Magill, T. Ahrens, and S. Morrisseau. 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298:2173-2176.

Metcalfe, A.C., M. Krsek, G.W. Gooday, J.I. Prosser, and E. M. H. Wellington. 2002. Molecular analysis of a bacterial chitinolytic community in an upland pasture. Applied and Environmental Microbiology 68:5042-5050.

Michalet, X.,F.F. Pinaud, L.A. Bentolila, J.M. Tsay, S. Doose, J.J. Li, G. Sundaresan, A.M. Wu, S.S. Gambhir, and S. Weiss. 2005. Quantum dots for live cells, in vivo imaging, and diagnostics. Science 307:538-544.

Michel, K., and E. Matzner. 2003. Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios., Biology and Fertility of Soils 38:102-109.

Mingardon, F., A. Chanal, C. Tardif, E.A. Bayer, and H.P. Fierobe. 2007. Exploration of new geometries in cellulosome-like chimeras. Applied and Environmental Microbiology 73:7138-7149.

Modak, T., S. Pradhan, and M. Watve. 2007. Sociobiology of biodegradation and the role of predatory protozoa in biodegrading communities. Journal of Biosciences 32:775-780.

Morrison, M., P.B. Pope,S.E. Denman, and C.S. McSweeney. 2009. Plant biomass degradation by gut microbiomes: more of the same or something new? Current Opinion in Biotechnology 20:358-363.

Nannipieri, P., 2006. Role of stabilised enzymes in microbial ecology and enzyme extraction from soil with potential applications in soil proteomics. Nucleic Acids and Proteins in Soil 8:75-94.

Nannipieri, P. and J. M. Bollag. 1991. Use of enzymes to detoxify pesticide-contaminated soils and waters. Journal of Environmental Quality 20:510-517.

Nannipieri, P., B. Ceccanti, and D. Bianchi. 1988. Characterization of humus phosphatase complexes extracted from soil. Soil Biology and Biochemistry 20: 683-691.

Nannipieri, P., B. Ceccanti, S. Cervelli,and P. Sequi. 1978. Stability and kinetic properties of humus urease complexes. Soil Biology and Biochemistry 10:143-148.

Nannipieri, P., E. Kandeler, and P. Ruggiero. 2002a. Enzyme activities and microbiological and biochemical processes in soil. p. 1-3 *In* R.G. Burns, R. P. Dick, (ed.) Enzymes in the Environment. Marcel Dekker, New York.

Niemi, R.M., and M. Vepsalainen. 2005. Stability of the fluorogenic enzyme substrates and pH optima of enzyme activities in different Finnish soils. Journal of Microbiological Methods 60:195-205.

Nigmatullin, R., R. Lovitt, C. Wright, M. Linder, T. Nakari-Setala, and A. Gama. 2004. Atomic force microscopy study of cellulose surface interaction controlled by cellulose binding domains. Colloids and Surfaces B-Biointerfaces 35:125-135.

Nozaki, M., C. Miura, Y. Tozawa, and T. Miura. 2009. The contribution of endogenous cellulase to the cellulose digestion in the gut of earthworm (Pheretima hilgendorfi: Megascolecidae). Soil Biology and Biochemistry 41: 762-769.

Nyanhongo, G.S., G. Gubitz, P. Sukyai, C. Leitner, D. Haltrich, and R. Ludwig. 2007. Oxidoreductases from Trametes spp. in biotechnology: A wealth of catalytic activity. Food Technology and Biotechnology 45:250-268.

Osono, T. 2007. Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecological Researc, 22:955-974.

Pang, Y., X. Liu, Y. Ma, L. Chernin, G. Berg, and K. Gao. 2009. Induction of systemic resistance, root colonisation and biocontrol activities of the rhizospheric strain of Serratia plymuthica are dependent on N-acyl homoserine lactones. European Journal of Plant Pathology 124:261-268.

Parham, J.A., and S.P. Deng. 2000. Detection, quantification and characterization of beta-glucosaminidase activity in soil. Soil Biology and Biochemistry 32:1183-1190.

Pastor, A., J. Chabert, M. Louwagie, J. Garin, and I. Attree. 920059. PscF is a major component of the Pseudomonas aeruginosa type III secretion needle. Fems Microbiology Letters 253:95-101.

Peer, A., S.P. Smith, E.A. Bayer, R. Lamed, and I. Borovok. 2009. Noncellulosomal cohesin- and dockerin-like modules in the three domains of life. Fems Microbiology Letters 291:1-16.

Pelletier, A., and J. Sygush. 1990. Purification and characterization of three chitosanase activities from *Bacillus megaterium* P1. Applied and Environmental Microbiology, 56:844-848.

Pflug, W. 1982. Soil enzymes and clay-minerals .2. Effect of clay-minerals on the activity of polysaccharide cleaving soil enzymes. Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 145:493-502.

Piccolo, A. 2001. The supramolecular structure of humic substances. Soil Science 166: 810-832.

Quiquampoix, H. and R.G. Burns. 2007. Interactions between proteins and soil mineral surfaces: Environmental and health consequences. Elements 3, 401-406.

Quiquampoix, H., S. Servagent-Noinville and M.H. Baron. 2002. Enzyme adsorption on soil mineral surfaces and consequences for the catalytic activity. p. 285-306. *In* R. Dick and R. Burns (eds) Enzymes in the Environment: Activity, Ecology, and Applications. Marcel Dekker, New York, New York, USA.

Raut, M. P., S. William, J.K. Bhattacharyya, T. Chakrabarti, and S. Devotta. 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid waste - A compost maturity analysis perspective. Bioresource Technology 99:6512-6519.

Romani, A. M., K. Fund, J. Artigas, T. Schwartz, S. Sabater, and U. Obst. 2008. Relevance of polymeric matrix enzymes during biofilm formation. Microbial Ecology 56:427-436.

Rubilar, O., M.C. Diez, and L. Gianfreda. 2008. Transformation of chlorinated phenolic compounds by white rot fungi. Critical Reviews in Environmental Science and Technology 38:227-268.

sandenoj 6/18/10 11:39 AM
Comment: In text but not in references

Matthew Wallenstein 6/20/10 8:53 PM
Deleted: Rubilar et al., 2008

Saiya-Cork, K.R., R.L. Sinsabaugh, and D,.R. Zak,. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology & Biochemistry 34:1309-1315.

Sanchez-Perez, G., A. Mira, G. Nyiro, L. Pasic, and F. Rodriguez-Valera. 2008. Adapting to environmental changes using specialized paralogs. Trends Genet: 24:154-158.

Schimel, J.P., and M.N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry 35:549-563.

Schwarz, W.H., and V.V. Zverlov. 2006. Protease inhibitors in bacteria: an emerging concept for the regulation of bacterial protein complexes? Molecular Microbiology 60:1323-1326.

Shackle, V., C. Freeman and B. Reynolds. 2006. Exogenous enzyme supplements to promote treatment efficiency in constructed wetlands. Science of the Total Environment 361:18-24.

Silva, A., M. Bacci, F.C. Pagnocca, O.C. Bueno, and M.J.A. Hebling,. 2006. Starch metabolism in Leucoagaricus gongylophorus, the symbiotic fungus of leaf-cutting ants. Microbiological Research 161:299-303.

Sinsabaugh, R.L. 1994. Enzymatic analysis of microbial pattern and process. Biology and Fertility of Soils ,17:69-74.

Sinsabaugh, R.L., M.M. Carreiro, and D.A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1-24.

Sinsabaugh, R.L., and D.L. Moorhead. 1994. Resource-allocation to extracellular enzyme-production - a model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26:1305-1311.

Skujins, J. 1978. History of abiontic soil enzyme research. p. 1-49 *In* R.G. Burns (ed) Soil Enzymes. Academic Press, London.

Steinweg, J.M., A.F. Plante, R.T. Conant, E.A. Paul, and D.L.Tanaka, Patterns of substrate utilization during long-term incubations at different temperatures. Soil Biology and Biochemistry 40:2722-2728.

Stursova, M., and R.L. Sinsabaugh. 2008. Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. Soil Biology and Biochemistry 40: 550-553.

Suarez, M. B., L. Sanz, M.I. Chamorro, M. Rey, F.J. Gonzalez, A. Llobell, and E. Monte. 200. Proteomic analysis of secreted proteins from Trichoderma harzianum - Identification of a fungal cell wall-induced aspartic protease. Fungal Genetics and Biology 42:924-934.

Tate, R.L. 2002. Microbiology and enzymology of carbon and nitrogen cycling. Enzymes in the Environment: Activity, Ecology, and Applications p. 227-248. *In* R. Dick and R. Burns (eds) Enzymes in the Environment: Activity, Ecology, and Applications. Marcel Dekker, New York, New York, USA.

Trasar-Cepeda, C., F. Gil-Sotres, and M.C. Leiros. 2007. Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. Soil Biology and Biochemistry 39:311-319.

Tsumuraya, Y. 1996. Expansins, nonenzymatic proteins promoting cell wall expansion in higher plants. Trends in Glycoscience and Glycotechnology 8:443-444.

Valaskova, V.,J. Snajdr, B. Bittner, T. Cajthaml, V. Merhautova, M. Hoffichter and P. Baldrian. 2007. Production of lignocellulose-degrading enzymes and degradation of leaf litter by saprotrophic basidiomycetes isolated from a Quercus petraea forest. Soil Biology and Biochemistry 39:2651-2660.

sandenoj 6/20/10 8:54 PM
Comment: Was this reference used? I do not seem to find it in the text YES- ON PAGE 15.

Vanden Wymelenberg, A.,G. Sabat, M. Mozuch, P.J. Kersten, D. Cullen, and R.A. Blanchette. 2006. Structure, organization, and transcriptional regulation of a family of copper radical oxidase genes in the lignin-degrading basidiomycete Phanerochaete chrysosporium. Applied and Environmental Microbiology 72:4871-4877.

Velicer, G.J. 2003. Social strife in the microbial world. Trends in Microbiology 11:330-337.

von Lutzow, M. and I. Kogel-Knabner. 2009. Temperature sensitivity of soil organic matter decomposition-what do we know? Biology and Fertility of Soils 46:1-15.

Wackett, L.P. 2008. Biomass to fuels via microbial transformations. Current Opinion in Chemical Biology 12:187-193.

Wackett, L.P. 2009. Questioning our perceptions about evolution of biodegradative enzymes. Current Opinion in Microbiology 12:244-251.

Waldrop, M.P., T.C. Balser, and M.K. Firestone. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology and Biochemistry 32: 1837-1846.

Wallenstein, M. D., S.K. McMahon, and J.P. Schimel. 2009. Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Global Change Biology 15:1631-1639.

Wallenstein, M.D., and M.N. Weintraub. 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biology and Biochemistry 40:2098-2106.

Whiteside, M.D., K.K. Treseder, and P.R. Atsatt. 2009. The brighter side of soils: Quantum dots track organic nitrogen through fungi and plants. Ecology 90:100-108.

Wilson, D.B. 2008. Three microbial strategies for plant cell wall degradation. Incredible Anaerobes: From Physiology to Genomics to Fuels 1125:289-297.

Wirth, S. J., and G.A. Wolf. 1992. Microplate Colourimetric Assay For Endoacting Cellulase, Xylanase, Chitinase, 1,3-Beta-Glucanase And Amylase Extracted From Forest Soil Horizons. Soil Biology and Biochemistry 24:511-519.

Witayakran, S., and A.J. Ragauskas. 2009. Modification of high-lignin softwood kraft pulp with laccase and amino acids. Enzyme and Microbial Technology 44:176-181.

Wong, D.W.S. 2009. Structure and Action Mechanism of Ligninolytic Enzymes. Appl. Biochem. Biotechnol 157:174-209.

sandenoj 6/21/10 12:34 PM
Comment: Really 35 pages? YES