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Review

Phenol oxidase, peroxidase and organic matter dynamics of soil

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ABSTRACT

Extracellular enzymes mediate the degradation, transformation and mineralization of soil organic matter. The activity of cellulases, phosphatases and other hydrolases has received extensive study and in many cases stoichiometric relationships and responses to disturbances are well established. In contrast, phenol oxidase and peroxidase activities, which are often uncorrelated with hydrolase activities, have been measured in only a small subset of soil enzyme studies. These enzymes are expressed for a variety of purposes including ontogeny, defense and the acquisition of carbon and nitrogen. Through excretion or lysis, these enzymes enter the environment where their aggregate activity mediates key ecosystem functions of lignin degradation, humification, carbon mineralization and dissolved organic carbon export. Phenol oxidases and peroxidases are less stable in the environment than extracellular hydrolases, especially when associated with organic particles. Activities are also affected, positively and negatively, by interaction with mineral surfaces. High spatiotemporal variation obscures their relationships with environmental variables and ecological process. Across ecosystems, phenol oxidase and peroxidase activities generally increase with soil pH, a finding not predicted from the pH optima of purified enzymes. Activities associated with plant litter and particulate organic matter often correlate with decomposition rates and potential activities generally increase with the lignin and secondary compound content of the material. At the ecosystem scale, nitrogen amendment alters the expression of phenol oxidase and peroxidase enzymes more broadly than culture studies imply and these responses correlate with positive and negative changes in litter decomposition rates and soil organic matter content. At the global scale, N amendment of basidiomycete-dominated soils of temperate and boreal forest ecoystems often leads to losses of oxidative enzyme activity, while activities in grassland soils dominated by glomeromycota and ascomycetes show little net response. Land use that leads to loss of soil organic matter tends to increase oxidative activities. Across ecosystems, soil organic matter content is not correlated with mean potential phenol oxidase and peroxidase activities. A multiple regression model that includes soil pH, mean annual temperature, mean annual precipitation and potential phenol oxidase activity accounts for 37% of the variation in soil organic matter (SOM) content across ecosystems ($n = 63$); a similar model for peroxidase activity describes 32% of SOM variance ($n = 43$). Analysis of residual variation suggest that suites of interacting factors create both positive and negative feedbacks on soil organic matter storage. Soils with high oxygen availability, pH and mineral activity tend to be substrate limited: high in situ oxidative activities limit soil organic matter accumulation. Soils with opposing characteristics are activity limited: low in situ oxidative activities promote soil organic matter storage.

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1. Introduction

Extracellular enzymes mediate the degradation, transformation and mineralization of soil organic matter. The activity of cellulases, phosphatases and other hydrolases has received extensive study and in many cases stoichiometric relationships and responses to disturbances are well established (Sinsabaugh et al., 2008). In contrast, phenol oxidase and peroxidase activities, which are often uncorrelated with hydrolase activities, have been measured in only a small subset of soil enzyme studies. These enzymes are expressed for a variety of purposes including ontogeny, defense and the acquisition of carbon and nitrogen. Through excretion or lysis, these enzymes enter the environment where their aggregate activity mediates key ecosystem functions of lignin degradation, humification, carbon mineralization and dissolved organic carbon export.

Within the soil biochemistry literature, enzymes that oxidize phenolic compounds using oxygen are often named in relation to a particular substrate (e.g. monophenol oxidase, tyrosinase, catechol

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oxidase, diphenol oxidase) even though the enzymes under consideration generally show activity to varying extents, against a broad range of molecules (Baldrian, 2006). The Enzyme Commission of IUPAC classifies these enzymes into several taxa. EC 1.10.3 includes enzymes that use oxygen as an electron acceptor. Within this group, EC 1.10.3.1 (o-diphenol: oxygen oxidoreductases) includes many enzymes described as tyrosinases or catechol oxidases and EC 1.10.3.2 (p-diphenol: oxygen oxidoreductases) includes laccases, which have multiple copper atoms at their reaction center. EC 1.13.11 includes enzymes that incorporate two oxygen atoms into their substrate (dioxygenases), e.g. EC 1.13.11.12 catechol dioxygenase. EC 1.14.18 includes enzymes that use an electron donor and incorporate a single oxygen into their substrate, e.g. EC 1.14.18.1 is defined as monophenol monooxygenase, a class that also encompasses enzymes variously described as tyrosinase, monophenol oxidase and laccase. Because assays of environmental samples may capture activity from some or all of these enzyme classes, I herein use the generic term "phenol oxidase" to describe the activity of enzymes that oxidize phenols and consume oxygen.

Peroxidases are enzymes that use H_2O_2 as an electron acceptor (EC 1.11.1). In soils, fungi produce manganese peroxidase (EC 1.11.1.13), lignin peroxidase (EC 1.11.1.14) and other broad spectrum peroxidases (EC 1.11.1.7) that are known for their role in depolymerizing lignin. As is the case for phenol oxidases, most assays of environmental samples do not discriminate individual enzymes, so herein I use the generic term "peroxidase" to describe the activity of enzymes that use H_2O_2 as an acceptor.

The first report of phenol oxidase activity in soil, using guaiacol as a substrate, was made by Cameron and Bell (1905, as cited in Skujins (1978)). Few studies followed. The text "Soil Enzymes" (Burns, 1978) included reviews of polysaccharidase, urease, phosphatase and sulfatase activities, but scant coverage and few references to phenol oxidase and peroxidase activities (Ladd, 1978). Studies by Mayaudon et al. (1973) and Ross and McNeilly (1973) increased interest in these activities, but 35 years later, I estimate that only about 150 papers have been published that include quantitative measurements from environmental samples.

2. Biochemistry

2.1. Phenol oxidases

Much of the research on fungal and bacterial degradation of aromatic compounds has been driven by biotechnical interests in the degradation of lignocellulose for pulp products and transportation fuels and the bioremediation of soils contaminated with pesticides, dioxins and other halogenated "xenobiotic" products. These topics are frequently reviewed (Duran et al., 2002; Claus, 2003; Rabinovich et al., 2004; Baldrian, 2006; Masai et al., 2007). Here, I include only a brief overview of this research to provide context for the synthesis of soil ecology studies that is the subject of this review.

Microorganisms and plants produce intracellular and extracellular phenol oxidases for a variety of purposes. Plants use phenol oxidases to synthesize lignin and other secondary compounds. Many fungi, but particularly ascomycetes and basidiomycetes use intracellular phenol oxidases to synthesize protective compounds like melanin, often in conjunction with spore formation or other morphogenic processes. Some organisms use extracellular phenol oxidases to degrade lignin and humus to gain carbon and other nutrients. More generally, extracellular phenol oxidases are deployed by both fungi and bacteria to mitigate the toxicity of phenolic molecules and metal ions, and aid in antimicrobial defense. But whatever their origin and initial function, phenol oxidases released into the environment whether by secretion or cell lysis are independent agents that catalyze non-specific reactions, including the oxidation of Mn^{+2} and Fe^{+2}, that can polymerize, depolymerize or transform a broad spectrum of phenolic molecules. These reactions, in turn, affect the activity and composition of soil microbial communities because phenolic molecules are inherently toxic.

The biochemistry of laccases, probably the largest class of phenol oxidases in soil, has been extensively reviewed (Duran et al., 2002; Claus, 2003; Rabinovich et al., 2004; Baldrian, 2006; Hoegger et al., 2006; Masai et al., 2007). Laccases are part of the multicopper oxidase protein family (Hoegger et al., 2006). Most enzymes have four copper atoms at their reaction center, and four single electron oxidations are needed to reduce oxygen to water. This stepwise process creates reactive semiquinones, quinones and phenoxy radicals. Laccases can also oxidize chelated Mn^{+2}. When used for lignin degradation, depolymerization is probably the result of diffusion of these reactive species rather than direct enzyme contact with the polymer.

Most basidiomycetes and many ascomycetes produce extracellular laccases. Based on analyses of more than 100 purified enzymes, fungal laccases have a median molecular size of 66 kD and median optimum temperature of 55 °C, but the range is wide for both values (Baldrian, 2006). Optimal pH values also vary widely because they depend on the substrate as well as the structure of the enzyme, but optima generally fall in the acidic range for extracellular enzymes, with more circumneutral values for intracellular enzymes. Extracellular laccases are glycoproteins with a typical saccharide content of 10–25%. Extracellular production can be constitutive or inducible with 1–8 isozymes produced per organism. Redox potentials range from 450 to 800 mV, less than that of lignin peroxidases (>1000 mV) but greater than that of catechol oxidases (200–300 mV).

Laccases can oxidize a broad range of small molecules including humics to form stable radicals, termed redox mediators, whose redox potentials are substantially greater than those of the laccases themselves (Leonowicz et al., 2001; Camarero et al., 2005). Many white rot fungi (Basidomycetes) lack peroxidases and depolymerize lignin using a system of laccase and redox mediators Laccases can also interact with Mn peroxidases by oxidizing chelated Mn^{+2}. In addition to lignin degradation, laccase production can be induced by presence of various phenols and as a component of a general antimicrobial defense. Production is also modulated by the availability of Cu.

Laccases are widely produced by white rot basidiomycetes and soft rot ascomycetes, but not by chytridiomycetes or zygomycetes (Bending and Read, 1997; Gunther et al., 1998; Lyons et al., 2003; Luis et al., 2005; Pointing et al., 2005; Kilaru et al., 2006; Zavarzina and Zavarzin, 2006; Kellner et al., 2008). Brown rot basidiomycetes produce intracellular laccases, which contribute to the degradation of lignocellulose upon cell lysis. The laccases of white rot fungi, generally have lower pH optima (4.0–5.0) than the laccases of brown rot and coprophilic fungi (6.0–7.5). The enzymes of the former group are primarily involved in lignin breakdown, often acting in concert with redox mediators, while the latter act primarily as detoxification agents, polymerizing soluble phenols and thereby contributing to humification. The laccases of ectomycorrhizal basidiomycetes do not cluster with the lignin-degrading laccases of white rot basidiomycetes (Courty et al., 2009). Some of these enzymes have ontogenetic roles, but others appear to function in concert with proteolytic and chitinolytic enzymes to extract nitrogen from humic complexes (Hobbie and Horton, 2007; Talbot et al., 2008; Courty et al., 2009).

Laccases are traditionally considered to be fungal enzymes. However, laccase genes, or more generally laccase-like multicopper oxidases (LMCO), are broadly distributed among bacteria and

archaea as are functionally similar enzymes that contain varying numbers and combinations of Cu, Mn, Zn or Fe atoms (Claus, 2003; Baldrian, 2006; Dalfard et al., 2006; Mayende et al., 2006; McMahon et al., 2007; Kellner et al., 2008; Li et al., 2008). Unlike fungi, bacterial LMCOs appear to components of larger protein complexes rather than independent agents (Claus, 2003). The diversity, and by extension activity, of bacterial LMCOs in soil may be much greater than those of fungi (Kellner et al., 2008). Fungi are generally considered the principal agents in initial degradation of plant litter, and lignin particularly, but most of the solubilized products of lignin degradation are probably metabolized by bacteria. A few bacteria, e.g. *Sphingomonas* (Alphaproteobacteria) are capable of lignin degradation using dehydrogenases to oxidize the dominant beta aryl ether linkage between phenylpropane units, and a variety of dioxygenases to remove methoxy groups and cleave rings (Masai et al., 2007).

2.2. Peroxidases

Some white rot (Basidiomycetes) and soft rot fungi (Ascomycetes) produce extracellular peroxidases in addition to phenol oxidases (for reviews see Higuchi, 1990; Hammel, 1997; Sinsabaugh, 2005; Rabinovich et al., 2004). Lignin peroxidase and Mn peroxidase have Fe heme prosthetic groups. Lignin peroxidase can directly oxidize the αC–βC bond between the phenylpropane units of lignin. Mn peroxidase attacks lignin indirectly by creating diffusable Mn^{3+}. The activity of these enzymes is supported by aryl alcohol oxidases (EC 1.1.3.7) that generate H_2O_2 by oxidizing hydroxyls to carbonyls and reducing oxygen to hydrogen peroxide. The saccharide-oxidizing enzymes glyoxal oxidase, galactose oxidase (EC 1.1.3.9) and glucose oxidase (EC 1.1.3.4), also reduce oxygen to H_2O_2, effectively coupling polysaccharide and lignin degradation. Cellobiose dehydrogenases (EC 1.1.99.18) reinforce this link by reducing phenoxy radicals, cations (Fe^{3+}, Cu^{2+}, and Mn^{3+}), or quinones using electrons from the oxidation of cellobiose to cellobionolactone, a process that also contributes to the availability of redox mediators.

Lignin peroxidase is induced by N deficiency in *Phanerochaete*, but this is not a general phenomenon. Peroxidase expression appears to be a response to oxidative stress, i.e. an increase in the concentration of reactive oxygen species, which can be created by various combinations of oxygen and nutrient availability (Rabinovich et al., 2004). Like phenol oxidases, enzyme expression is also induced by various phenolic compounds, suggesting that these enzymes also play a role in detoxification. Mn availability also regulates peroxidase expression. High Mn availability increases oxidative stress and also shifts expression toward Mn peroxidase, rather than lignin peroxidase, production.

2.3. Mineral catalysis

In addition to enzymatic reactions, the mineral matrix of soil itself contributes to its oxidative potential. Any abiotic process that generates reactive oxygen species or other radicals contributes to the oxidative transformation of organic matter. A well known example is the Fenton reaction in which Fe catalyzes the conversion of H_2O_2, generated by aryl alcohol or saccharide oxidases, to hydroxyl radicals that can break aromatic rings (Rabinovich et al., 2004). Non-phenolic linkages in lignin can also be broken by Mn^{+3} which can be generated by both laccases and Mn peroxidases. Mn^{+3} can also peroxidate unsaturated lipids, creating a secondary redox mediator. In general, oxides and hydroxides of Mn, Fe and Al as well as silicate clays contribute to humification reactions by providing binding sites and electron acceptors that catalyze the formation of semiquinones and other reactive species from phenols. These reactive intermediates condense with other phenols or amino acids to produce humic compounds (Boyd and Mortland, 1990; Huang, 1990; Nannipieri et al., 2002). Reaction rates vary widely in relation to mineral composition, specific surface area and pH. Mn oxides have the greatest activity, but the silt and clay fractions of almost any soil catalyze decarboxylation and deamination reactions that contribute to the mineralization of organic matter and condensation reactions that lead to the formation of humic acids (Huang, 1990).

2.4. Ecosystem perspective

As the preceding sections make clear, perhaps the primary difficulty in interpreting measurements of the oxidative potential of soils is that this activity is associated with at least four major processes: synthesis of secondary compounds, decomposition, defense and humification. These processes cannot be resolved at the community or ecosystem scale using simple metrics. Nevertheless, abstracted to the ecosystem scale the net effect of this oxidative activity is that all or nearly all of the lignin and other secondary compounds that enter the system is eventually mineralized. In some systems, a small portion of the input may accumulate as soil organic matter or be exported as dissolved organic matter.

3. Assay methods

Phenol oxidases and peroxidases mediate non-specific free-radical reactions over a wide range of redox potential, and often in conjunction with secondary mediators, both organic and inorganic. Consequently, assays that use a single model substrate may capture only a fraction of the oxidative potential resident in environmental samples. In cell-free or culture systems a variety of substrates are used to quantify and discriminate various enzymatic activities. Baldrian (2006) compiled kinetic data for purified fungal laccases against ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), DMP (2,6-dimethoxyphenol), guaiacol (2-methoxyphenol) and syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde). Of these, activity against syringaldazine is generally considered a defining feature of laccases. For lignin peroxidase, the oxidation of veratryl alcohol (3,4-dimethoxybenzyl alcohol) to veratraldehyde is considered the standard assay (Hammel, 1997). Mn peroxidase activity is often assayed using o-dianisidine (3,3′-dimethoxybenzidine) (Sklarz et al., 1989). None of these substrates are typically used to assay oxidative enzyme activity in soils because of their limited solubility in aqueous solution, sorption to soil colloids (Claus and Filip, 1990), or because reaction products cannot be quantified against high background absorbance by soil humics (Nanniperi et al., 1991).

Early studies of phenol oxidase activity in soils used oxygen consumption as a measure. This assay has largely been displaced by more convenient colorimetric assays. The substrate most widely used to measure phenol oxidase activity in soil assays is DOPA (L-3,4-dihydroxyphenylalanine) because it is inexpensive, reasonably soluble (>20 mM) and produces oxidation products that can be detected in the visible spectrum (450 nm) (Sinsabaugh and Linkins, 1988; Pind et al., 1994; Sinsabaugh et al., 2008). The DOPA assay is far from ideal. It has been criticized for being too sensitive (o-diphenols have low redox potentials) and not sensitive enough (in direct comparison to other substrates). Other problems include susceptibility to chemical oxidation by Mn and Fe species and the potential for competitive reactions that consume oxidation products, thereby limiting color development. For these reasons, the rate of DOPA oxidation in particular soils may not vary linearly with time, substrate concentration or soil dilution.

There have been several efforts to develop alternative assays to mitigate some of these problems. Nanniperi et al. (1991) presented

an assay that uses guaiacol as a substrate. The assay has a pH optimum of 4.5–6.0, compared an optimum pH of 8.0–9.6 for DOPA (Pind et al., 1994). Guaiacol disappearance was quantified by measuring the change in absorbance at 275 nm. Because aromatic compounds have strong absorbance in the UVB range, guaiacol must be extracted and purified by liquid chromatography to get results. Perucci et al. (2000) developed an assay that quantifies the oxidation of catechol by measuring the formation of a secondary condensation product from the reaction of the intermediate quinone with proline. The assay showed a pH optimum of 6.5–7.5 and yielded higher reaction rates than DOPA. Luis et al. (2005) used ABTS to measure laccase activity in beech-oak cambisols. A more thorough evaluation of ABTS was presented by Floch et al. (2007). The assay proved sensitive and free of interference. It is interesting to note that the soils tested showed no activity against syringaldazine or 2,7 -diaminofluorene which like ABTS are common laccase substrates.

In several studies by Allison and Vitousek (beginning 2004), pyrogallol (1,2,3-trihydroxybenzene) buffered to pH 5 was used as a substrate for phenol oxidase assays. Fioretto et al. (2000) and Nardo et al. (2004) used o-toluidine (3,3′ dimetyl 4,4′ diamino biphenyl) buffered at pH 5 to assay both phenol oxidase and peroxidase activities. Benitez et al. (2006) measured catechol oxidation at pH 6.5.

All of these assays share some common features. First, the "best" results are obtained at high soil dilutions ($\sim 10^{-2}$) presumably because interference from competing reactions is minimized. Second, all generate similar ranges for soil phenol oxidase (typically 1–50 μmol h^{-1} g^{-1} with spikes to 300), whether activities are measured using guaicol, catechol, ABTS, pyrogallol, catechol or o-toluidine as substrates. A compilation of phenol oxidase activities, measured using the DOPA assay, shows a similar range of mean values and extremes (0–34 μmol h^{-1} g^{-1} with spikes 300, Sinsabaugh et al. (2008)). This convergence suggests that substrates with similar redox potentials generate similar rates and that our methodologies may be less problematic than we often assume, although particular substrates may have advantages in specific cases.

Soil peroxidase activity is most commonly measured by adding DOPA and H_2O_2 to soil suspensions and comparing the results to (phenol oxidase) assays that receive DOPA alone. This assay is subject to all the shortcomings of the phenol oxidase assay plus the added uncertainty of competing reactions for H_2O_2. The range of values resported for soil peroxidase activity is similar to that for phenol oxidase (Sinsabaugh et al., 2008). Fioretto et al. (2000) and Nardo et al. (2004) used o-toluidine with H_2O_2 to assay peroxidase associated with decomposing leaf litter, yielding values similar to those reported for litter using DOPA. Johnsen and Jacobsen (2008) proposed a new assay for soil peroxidase that quantifies the oxidation of TMB (3,3′,5,5′-tetramethylbenzidine) at pH 4.4. In tests with nine soils, there was little TMB oxidation in the absence of added H_2O_2 and TMB was more sensitive than DOPA (which showed net peroxidase activity in only two of the soils). Because TMB oxidation rates were reported as change in absorbance at 450 nm, rather than change in substrate concentration, quantitative comparisons with other substrates cannot be made.

Many of the papers that report on methods for measuring oxidative enzyme activities in soil recommend using autoclaved soil as a control. The rationale is that enzymes will be denatured by autoclaving, thus any residual oxidation of added substrate will the a product of chemical catalysis by mineral colloids. This generalization may not hold for all soils. In some cases, enzymes bound to mineral complexes retain activity even after autoclaving (Stursova and Sinsabaugh, 2008). Another consideration is that continuing research on lignin degradation and humification shows that these processes may involve complex, and potentially synergistic, interactions between enzymes and mineral surfaces that promote oxidative activity through redox mediators and catalytic cycles (e.g. Naidja et al., 1995; Allison, 2006). Under such circumstances, the gross activity of untreated soils may be a more robust measure of soil oxidative potential, than the net difference in activity between untreated and autoclaved samples.

4. Spatiotemporal variation

Measurements of soil phenol oxidase and peroxidase activities show great spatiotemporal variation, in both absolute terms and in relation to soil hydrolase activities. In a comparison of forty soils from diverse biomes, the coefficient of variation for mean phenol oxidase and peroxidase activities among ecosystems was nearly 300%, compared to values 70% for β-glucosidase and phosphatase (Sinsabaugh et al., 2008). Within ecosystems, coefficents of variation for phenol oxidase and peroxidase activities ranged from 30% to >300%.

In temperate climates, variation may have a seasonal component. Some studies show that activity in forest litter and mineral soils dips during the summer and may become undetectable (Criquet et al., 2000; Sinsabaugh et al., 2005; Finzi et al., 2006; Weintraub et al., 2008), probably as a result of moisture limitation (Toberman et al., 2008a) or in some cases changes in carbon availability. But other studies have found no significant seasonal variation (Sinsabaugh et al., 2003; Boerner et al., 2005; Kellner et al., 2009). In peatlands, seasonal drought has been reported to have positive (Nathalie et al., 2005) or neutral (Freeman et al., 1996) on phenol oxidase activity, reflecting the interaction of oxygen and water availability.

Decker et al. (2005) examined the spatial variation of soil enzyme activities in temperate oak forests over scales from meters to kilometers. Hydrolase activities generally correlated with soil organic matter (SOM) and nutrient availabilities while phenol oxidase activity correlated with soil pH and was the more difficult to model.Snajdr et al. (2008) looked at smaller scale variation in microbial communty composition and enzyme activity in oak forest soils: Peroxidase activities varied more than 15X on horizontal scales of 50 cm and was significantly greater in the H horizon than the L horizon, which were separated by only 1 cm. Sedia and Ehrenfeld (2006) found that ground cover by lichen and moss was correlated with soil phenol oxidase in New Jersey pine barrens. Mora et al. (2005) found that the highest enzyme activities including phenol oxidase, in a neotropical savannah were associated with biogenic structures, particularly wormcasts.

Within the soil profile, activity generally declines with soil depth (Luis et al., 2005), and the highest mass-specific activities are generally associated with the silt-clay fraction (Grandy et al., 2008; Matocha et al., 2004; Henry et al., 2005; Allison and Jastrow, 2006). But there are exceptions. Stursova and Sinsabaugh (2008) found that phenol oxidase and peroxidase activities were similar for ten size ranges of particles from >1 mm to <38 μm in a Typic Haplargid from an arid grassland. Jackson et al. (2009) found that peroxidase activity, unlike hydrolytic activities, increased with depth in tropical peat soils.

In general, we expect microbial activity including enzyme activities to vary with temperature, moisture, pH and other physicochemical variables as well as in relation to carbon and nutrient availability. What distinguishes phenol oxidase and peroxidase activities is that the magnitude of the variation exceeds that of hydrolase activities and often there is little correlation between hydrolytic and oxidative activities (Sinsabaugh et al., 2008). Principal components or multidimensional scaling analyses of soil and litter enzyme activity typically condense oxidative and hydrolytic activities onto separate axes (Saiya-Cork, 2002; Sinsabaugh et al., 2002, 2004, 2005; Stursova et al., 2006; Zeglin et al., 2007; Henry et al., 2005).

This dichotomy suggests that the controls on microbial enzyme expression and environmental enzyme turnover differ for these two broad classes of enzymes. Factors known to influence phenol oxidase and peroxidase activities in soils include the concentration of soluble phenolic compounds, the lignin content of plant litter, soil pH, and nitrogen availability. In response to these variables, oxidative and hydrolase activities tend to shift in opposite directions. However, oxidative activities are generally more dynamic which suggest that these enzymes are less stable in the soil environment than many hydrolases.

5. Environmental stability

The case for higher turnover rates of phenol oxidases and peroxidases in the soil environment is largely circumstantial. Mean spatiotemporal variation in activity within ecosystems is approximately three times greater than that of hydrolases (Sinsabaugh et al., 2008). The median molecular size for fungal laccases (66 kD, Baldrian, 2006) is about twice that for most cellulases and phosphatases. Greater complexity of structure may offer greater opportunity for inactivation.

Phenol oxidases and peroxidases are subject to the same non-competitive reactions and proteolytic attack as hydrolases, but are exposed to added threats from their own reaction products (Gianfreda and Bollag, 1994; Duran et al., 2002) and chelators that may pull Fe and Cu atoms from active sites (Boyd and Mortland, 1990; Huang, 1990; Keum and Li, 2004). Gianfreda and Bollag (1994) found that the activity of laccase and peroxidase enzymes immobilized on glass, montmorillonite, and kaolinite was stable indefinitely, but declined markedly when mixed with soil in proportion to its organic matter content. Allison (2006) studying gamma irradiated volcanic soil found that the turnover rate for phenol oxidase was 20X faster than that of β-glucosidase (0.628 vs 0.032/d). Additions of allophane and ferrihydrite increased phenol oxidase activity while humic acid addition reduced it.

The behavior of immobilized enzymes in engineered reactors and soils suggests that the turnover rate for phenol oxidases and peroxidases may vary in relation to soil organic matter content and climate. Low soil organic matter and dry conditions may stabilize activity in arid environments (Stursova and Sinsabaugh, 2008), while wetter climate and high humus content may reduce effective enzyme life. A related conjecture is that drying down mineral soil with immobilized enzymes may stabilize a large fraction of the associated activity while drying down organic particles leads to significant losses of associated oxidative activities. The latter effect is consistent with observations noted previously that litter-associated phenol oxidase and peroxidase activities drop precipitiously during dry seasons. Similar declines have also been observed for drying peats (Laiho, 2006). Repeated wet–dry cycles also tend to promote humification (polymerization) reactions over solubilization (Huang, 1990). These phenomena suggest that the dynamics of phenol oxidase and peroxidase enzymes in relation to edaphic and climate factors may be an important mechanism underlying patterns soil organic matter accumulation.

6. Soil pH effects

Hydrolases and other enzymes that act on a narrow range of substrates have pH optima defined largely by their reaction mechanism. The non-specific reactions catalyzed by phenol oxidases and peroxidases are less constrained. Their reaction centers contain Cu, Fe or other metal atoms whose ability to transfer electrons is affected by ambient pH, but mediated by surrounding amino acid side chains. For fungal laccases, optimal pH for activity against a specific substrate, e.g. ABTS, while generally acidic to neutral varies among enzymes by several units (Baldrian, 2006; Li et al., 2008). Moreover, for individual enzymes, optimal pH for activity may vary in relation to substrate over several pH units (Baldrian, 2006; Li et al., 2008). This phenomenon extends to soil enzyme pools as well where optimal pH values for phenol oxidase and peroxidase assays range from 4 to 5 for TMB and guaiacol to 8–9 for DOPA. Given the diversity of enzymes in the soil, the broad spectrum of potential mediators and reactants, and other conflating factors, the role of bulk soil pH in regulating aggregrate oxidative activity is not clear. Within biomes, phenol oxidase activity increases with pH (Li et al., 2008; Williams et al., 2000; Decker et al., 1999). Based on the DOPA assay, Sinsabaugh et al. (2008) found that phenol oxidase and peroxidase activities in 35 soils increased significantly with soil pH, whether expressed on a dry mass or organic matter specific basis. An updated analysis that includes 67 soils shows that both phenol oxidase and peroxidase activities per g soil increase by about one $\log_e$ unit (2.7X) for each unit increase in pH (Fig. 1). Hydrolase activities also vary in relation to soil pH, but also show significant relationships with soil organic matter content and climate variables, unlike oxidative activities (Sinsabaugh et al., 2008).

The generally positive relationship between potential phenol oxidase and peroxidase activities and soil pH is consistent with a number of other chemical and microbial trends. As pH increases, phenols deprotonate which reduces their redox potential and increases their solubility both of which may enhance their reaction potential. Another factor is that soil enzymes are mostly immobilized on colloids and sorption of enzyme to clays and organic particles generally displaces their apparent pH optimum 1–2 units toward alkaline because surface pH is lower than that of soil solution (Sinsabaugh and Linkins, 1987; Boyd and Mortland, 1990; Huang, 1990, Nannipieri et al., 2002). Finally, recent studies identify pH as a prominent predictor of microbial diversity (Fierer and Jackson, 2006; Lauber et al., 2008, 2009a; Hartman et al., 2008).

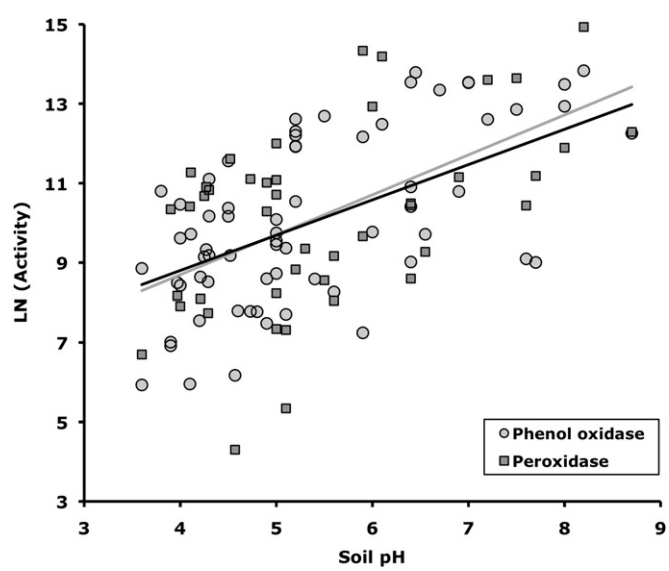

Fig. 1. Phenol oxidase and peroxidase activities (nmol h^{-1} g^{-1} soil) in relation to soil pH. Data points are mean values for individual ecosystems. For phenol oxidase (light line), $n = 67$, $R^2 = 0.37$, LN(activity) = 1.006(pH) + 4.67. For peroxidase (dark line), $n = 44$, $R^2 = 0.25$, LN(activity) = 0.889(pH) + 5.25. The data include 35 soils described by Sinsabaugh et al. (2008) plus additional data from Boerner and Brinkman (2003), Boerner et al. (2005), Toberman et al. (2008a,b), Frey et al. (2004), Lucas et al. (2007), Lyyemperumal and Shi (2008), Matocha et al. (2004), Henry et al. (2004), Allison and Jastrow (2006), Allison et al. (2006), Sedia and Ehrenfeld (2006), Fenner et al. (2005a, b), Stursova and White (unpubl.), Li et al. (2006), Williams et al. (2000), Benitez et al. (2006), Waldrop et al. (2000), Waldrop and Harden (2008), and Keeler et al. (in press).

Because phenol oxidases are widely produced for varied purposes with little conserved homology, it is plausible that the diversity of the soil enzyme pool and potentially its range of action may also expand with soil pH.

7. Metagenomics

Because of their mediating role in soil organic matter dynamics there is growing interest in the metagenomics and metaproteomics of phenol oxidases. Luis et al. (2004, 2005) looked at spatial heterogeneity in the diversity of basidiomycete laccase genes in a beech-oak forest soil in Germany. Diversity was greatest in the organic horizon and decreased with soil depth, paralleling a decrease in phenol oxidase activity, and there was 67% dissimilarity between adjacent cores separated by 30 cm. A total of 167 putative laccase genes were found but rarefaction analysis suggested that these represented only half the total richness. Of the 108 sequences that could be linked to specific taxa, two-thirds were associated with ectomycorrhizal fungi, the remainder to saprotrophs.

A follow up study (Kellner et al., 2009) focused on temporal variation in laccase gene richness and expression within beech litter sampled bimonthly. A total of 73 basidiomycete laccase genes were detected (21 from saprotrophs, 22 from mycorrhizal fungi, rest unknown). From cDNA libraries of mRNA extracts, they recovered 42 laccase genes, only five of which matched the genes from DNA extracts. Ascomycete laccase primers did not work for DNA extracts, but among the 42 expressed laccase genes 28 were basidiomycete and 14 were ascomycete. Laccase diversity peaked with autumn litter fall, but phenol oxidase activity in the litter was similar across seasons.

The distribution of basidiomycete laccase genes has also been examined in temperate forest sites in northern Michigan. Blackwood et al. (2007) using quantitative PCR found that the diversity of basidiomycetes was similar in organic horizons of forests dominated by sugar maple and oaks but basidiomycete abundance was about 10X greater in the oak forest litter. In response to experimental nitrogen enrichment, phenol oxidase activity declined sharply in the oak forest litter relative to control sites, but the abundance and diversity of basidiomycetes and basidiomycete laccase genes was unaffected. Using a similar approach, Hofmockel et al. (2007) found that basidiomycete laccase gene abundance was 2.5X greater in surface litter than in upper mineral soil, but also found no correlation between gene abundance and phenol oxidase activities. Lauber et al. (2009b) focused on the upper mineral soil horizon of the oak forest sites and likewise found no change in either the composition or abundance of basidiomycete laccase genes, despite a 50% decline in soil phenol oxidase activity in the N enriched plots. About 55% of the soil laccase genes were associated with ectomycorrhizal fungi. A principal components analysis (Unifrac) showed that the laccase metagenome of the oak soil was more similar to that reported by Luis et al. (2004, 2005) than it was to the overlying litter metagenome, which was dominated by saprotroph genes (Blackwood et al., 2007). Artz et al. (2009) found 95 basidiomycete laccase genes in the soil of Australian Eucalytpus forests subjected to different fire management regimes. These genes clustered with sequences compiled by Hoegger et al. (2006).

Sampling some of the same beech-oak sites as Luis et al. (2005), Kellner et al. (2008) surveyed bacterial laccase genes. The 121 genes grouped into 16 clades of which only half matched a known bacteria. Rarefaction analysis showed no inflection toward saturation. Within the same forest soils, Luis et al. (2005) found only 63 basidiomycete genes. Samples from a grassland site yielded 22 bacterial genes, mostly rhizobiales, and no basidiomycete sequences.

Lyons et al. (2003) amplified ascomycete laccase genes from cultures isolated from *Spartina alterniflora* litter decomposing in a salt marsh. A total of 39 distinct genes were detected and some cultures had multiple genes. DNA extracts from the litter yielded seven sequences, five of which matched with those obtained from culture.

The limited work to date suggests that soil metagenomes contain hundreds to thousands of laccase and other phenol oxidase genes broadly distributed across both symbiotic and saprotrophic fungi and bacteria. On large scales, this metagenome shifts with canopy and litter composition and the shift from ectomycorrhizal to arbuscular mycorrhizal symbioses, but the implications of this for the regulation of soil oxidative activity in relation to global shifts in climate or N deposition are largely unknown (Talbot et al., 2008).

8. Surface litter decomposition

Most studies that include measurement of phenol oxidase or peroxidase activities associated with plant litter compare O horizon samples collected across experimental units or environmental gradients. Only a few studies have followed these activities over the course of decomposition for defined litter cohorts.

Sinsabaugh et al. (1992a,b) measured EEA associated with birch (*Betula papyfera*) sticks decomposing in stream, riparian and forest floor habitats in a temperate forest. Phenol oxidase activity generally increased with time and was strongly correlated with cellulolytic activities. As a result a single factor, cumulative "lignocellulase activity" accounted for over 90% of the variation in mass loss rates among habitats.

Fioretto et al. (2000) followed phenol oxidase, peroxidase and other extracellular enzyme activities (EEA) for decomposing leaves of rockrose (*Cistus icanus*) and myrtle (*Myrtus communis*) in a Mediterranean shrubland. Phenol oxidase activity was strongly correlated with fungal mass. Peroxidase activity was found only on *Myrtus* litter.

Sinsabaugh et al. (2002) calculated turnover activities, i.e. the total activity potential needed to decompose the litter cohort, for dogwood (*Cornus florida*), maple (*Acer rubrum*) and oak (*Quercus borealis*) leaves decomposing in a temperate forest site. For phenol oxidase, turnover activities increased exponentially from dogwood to maple to oak in a ratio of 1:2:7.5; following a trend of increasing litter LCI (lignocellulose index = lignin/(lignin + cellulose)) (Table 1). The corresponding ratio for peroxidase (1:5:2) was more closely related to soluble phenol content and fungal biomass.

Waldrop et al. (2003) compared the decomposition of *Pinus ponderosa* litter in undisturbed forest to that of logged sites subjected to three types of post-harvest treatment in the Sierra Nevada (CA, USA). Phenol oxidase activity and mass loss rates were greatest at the undisturbed site. Activity and mass loss rate declined with the intensity of the post-harvest treatment.

Allison and Vitousek (2004) followed decomposition of 15 species of tropical litter in control and N fertilized plots in Hawaii. Across litter types, mass loss rates were positively correlated with phenol oxidase and cellobiohydrolase activities, but more strongly with soluble carbon content (mean annual precipitation at the site exceeds 4 m). In relation to N fertilization, five litter species decomposed more rapidly, ten showed no response, and cumulative phenol oxidase activities did not change significantly.

Nardo et al. (2004) measured phenol oxidase and peroxidase activities associated with decomposing oak litter (*Quercus ilex*) in a Mediterranean shrubland. Enzyme activation energies varied seasonally ranging from 15 to 60 kJ/mol. Activities showed a strong seasonal pattern with winter highs and summer lows.

Harner et al. (2009) followed the decomposition of cottonwood (*Populus deltoides*) and Russian olive (*Elaeagnus angustifolia*, an actinorhizal species) litter in riparian surface, riparian subsurface

Table 1
Turnover activities (T) for phenol oxidase (POX) and peroxidase (PER). Turnover activities (mmol/g OM) are calculated from regressions of temporally integrated enzyme activity as a function of mass loss. For reference, the carbon content of organic matter is approximately 38 mmol/g.

System	T_{POX}	T_{PER}	Reference
Sticks in lotic, riparian and upland forest habitats			Sinsabaugh et al., 1992a,b
Betula papyfera sticks	72		
Wood in small stream			Hendel and Marxsen, 2000
Fagus sylvatica sapwood		24	
Wood in small streams			Tank et al., 1998
Liriodendron tulipifera strips	26	15	
Stream particulate organic matter			Sinsabaugh et al., 1994
Coarse 1–4 mm	27	48	
Medium 0.25–1 mm	103	181	
Fine 0.063–0.25 mm	145	333	
Leaf litter in temperate forest			Sinsabaugh et al., 2002
Cornus florida	127	32	
Acer rubrum	257	161	
Quercus borealis	953	66	
Wetland particulate organic matter			Jackson et al., 1995
Coarse 1–4 mm	58	370	
Medium 0.25–1 mm	435	2000	
Fine 0.063–0.25 mm	370	1430	
Leaf litter in semiarid riparian zone			Gallo et al., 2009
Populus deltoides	7	7	
Juniperis monosperma	3	6	
Pinus edulis	5	5	
Foliar litter in tropical forest			Allison and Vitousek, 2004
Cibotium glaucum stem		38	
Dicranopteris linearis stem		75	
Sphaeropteris cooperi stem		55	
Hedychium gardarianum stem	14		
Cibotium glaucum frond		60	
Dicranopteris linearis frond		76	
Diplazium sandwichianum frond		146	
Elaphoglossum alatum frond	178		
Sphaeropteris cooperi frond		27	
Vaccinium calycinum leaf		39	
Hedychium gardarianum leaf	75		
Setaria palmifolia leaf	56		
Clidermis hirta leaf		30	
Miconia calvescens leaf	35		
Tibouchina herbacea leaf		27	
Leaf litter in Mediterranean shrubland			Fioretto et al., 2000
Cistus icanus (under Cistus)		170	
C. icanus (under Myrtus)	290		
Myrtus communis (under Myrtus)		615	
Leaf litter in oak shrubland			Nardo et al., 2004
Quercus ilex	780	580	
Pinus ponderosa litter in pine forest			Waldrop et al. (2003)
Litterbags: Intact forest	68		
Slash post-harvest	57		
Chip&pile post-harvest	52		
Broadcast&burn post-harvest	77		
Forest floor: Intact forest	262		
Slash post-harvest	108		
Chip&pile post-harvest	180		
Broadcast&burn post-harvest	149		

and river channel habitats along the middle Rio Grande (New Mexico, USA). In general, mass loss rates were proportional to enzymatic activity, which in turn was linked to fungal biomass, which increased with litter nitrogen content. Oxidative activities were similar for the two litter types. But there was a habitat difference: peroxidase activity associated with litter submerged in the river channel was only half that measured in the terrestrial litter.

In another study along the middle Rio Grande, Gallo et al. (2009) followed decomposition of cottonwood (*P. deltoides*), juniper (*Juniperus monosperma*) and piñon (*Pinus edulis*) leaf litter in relation to sunlight exposure. Turnover activities for phenol oxidase and peroxidase were an order of magnitude lower than those calculated from other studies (Table 1). Activities did not vary with litter type. Molecular analyses showed that the litter-associated fungal communities were dominated by taxa, mostly ascomycetes, considered to be "early successional" colonists rather than lignocellulose degraders. In this arid environment, photoreactions supplement enzymatic reactions (Brandt et al., 2009). As a result mass loss rates are similar to those of mesic ecosystems even though turnover activities are much lower.

Because litter decomposition leads to comminution, particle size has been used as a surrogate for processing time, particularly in aquatic ecosystems. In these systems, phenol oxidase and peroxidase activities per g OM tend to increase as particle size declines. Mean values, especially for particles <1 mm, are similar to those tabulated by Sinsabaugh et al. (2008) for bulk soils (Fig. 2).

Across studies, estimated phenol oxidase turnover activities for foliar litter and particulate organic matter range from 3 to 973 mmol/g (Table 1). The lowest values (Gallo et al., 2009) come from a desert system with high photodegradation rates. The greatest values are associated with fine particulate organic matter (<1 mm) and litter with high contents of lignin or phenolics, e.g oak and myrtle species. Peroxidase turnover activities have a larger range (5–2000 mmol/g), but the number of estimates is small. While it is clear that both environmental variables and litter characteristics affect these values, there are not enough data to isolate the role of individual variables.

9. Soil activity

On a global basis, phenol oxidase and peroxidase activities in bulk soil have been related to soil pH, mean annual precipitation and mean annual temperature (Sinsabaugh et al., 2008). On the ecosystem scale, activities have been compared in a variety of contexts, most commonly land management practice and global change scenarios.

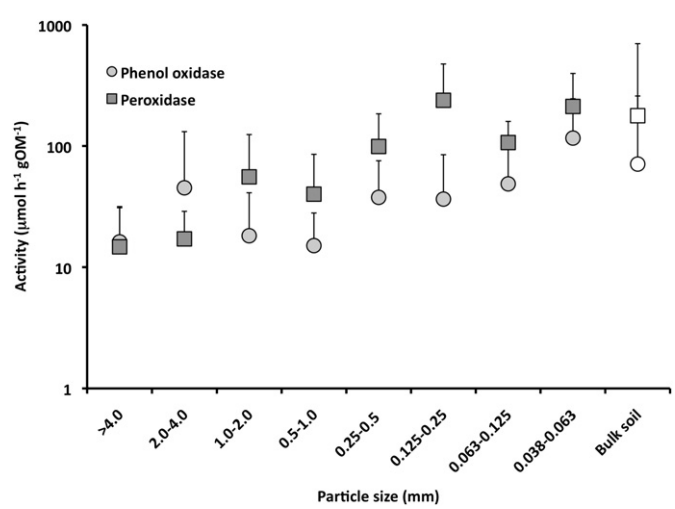

Fig. 2. Mean phenol oxidase and peroxidase activities (+SD) for particulate organic matter in aquatic ecosystems. Mean values for bulk soil (Sinsabaugh et al., 2008) are shown for reference. Data are pooled from studies by Sinsabaugh and Linkins (1990), Sinsabaugh et al. (1992a,b, 1994), Sinsabaugh and Findlay (1995), Jackson et al., 1995, Alvarez and Guerrero (2000).

Table 2
Effect of inorganic nitrogen enrichment on phenol oxidase and peroxidase activities in litter and soil. Effects are expressed as a response ratio (R, treatment/control). In cases where the experimental manipulation included two or more levels of enrichment, R is given for all treatments beginning with the lowest level. Superscripts indicate studies performed at the same experimental sites.

System	POX	PER	Reference
Black/White oak forest soil, MI			[1]Grandy et al., 2008
>250 mm	1.04	0.93	
63–250 mm	0.56	0.76	
<63 mm	0.83	0.92	
Sugar maple/basswood forest soil, MI			[1]Grandy et al., 2008
>250 mm	0.56	0.54	
63–250 mm	0.71	0.61	
<63 mm	0.86	0.89	
Sugar maple/basswood forest, MI			[1]Sinsabaugh et al., 2005
Litter	0.65/0.54	1.23/1.38	
Soil		1.08/1.03	
Sugar maple/red oak forest, MI			[1]Sinsabaugh et al., 2005
Litter	0.70/0.65	1.13/0.85	
Soil	0.49/0.18	0.76/0.71	
Black/White oak forest, MI			[1]Sinsabaugh et al., 2005
Litter	1.13/0.48	1.05/0.77	
Soil	0.24/0.53	0.79/0.61	
Sugar maple/basswood forest, MI			[1]Waldrop et al., 2004
Soil	1.40	1. 31	
Sugar maple/red oak forest, MI			[1]Waldrop et al., 2004
Soil	1.14	1. 03	
Black/White oak forest, MI			[1]Waldrop et al., 2004
Soil	0.81	0.90	
Sugar maple/basswood forest, MI			[1]Hofmockel et al., 2007
Litter	1.00/2.00		
Soil	0.50/0.50		
Sugar maple/red oak forest, MI			[1]Hofmockel et al., 2007
Litter	0.50/0.35		
Soil	2.33/0.50		
Black/White oak forest, MI			[1]Hofmockel et al., 2007
Litter	1.14/0.71		
Soil	0.62/0.46		
Sugar maple/basswood forest, MI			[1]Gallo et al., 2004
Litter	1.08/1.27	0.90/0.87	
Soil	1.01/0.27	0.65/0.66	
Sugar maple/red oak forest, MI			[1]Gallo et al., 2004
Litter	0.86/0.98	1.57/1.60	
Soil	1.71/0.67	1.11/0.95	
Black/White oak forest, MI			[1]Gallo et al., 2004
Litter	1.23/0.95	1.17/1.17	
Soil	0.35/0.45	0.64/0.59	
Sugar maple/red oak forest, MI			[2]Saiya-Cork et al., 2002
Litter	1.63	0.93	
Soil	0.60	1.07	
Sugar maple/red oak forest, MI			[2]DeForest et al., 2004a
Litter	0.65	0.68	
Soil	0.95	0.85	
Sugar maple/red oak forest, MI			[2]DeForest et al., 2004b
Soil	0.17	0.26	
Hardwood forest soil, MA	0.60/0.52		Frey et al., 2004
Red pine forest soil, MA	0.71/0.72		Frey et al., 2004
Hardwood forest, NY			Carreiro et al., 2000
Dogwood litter	0.40/1.50	0.90/0.88	
Red maple litter	1.15/1.05	0.95/0.95	
Red oak litter	0.65/0.46	1.15/1.25	
Pine forest, NJ			Lucas et al., 2007
Soil	1.00/1.40	0.50/0.46	
Red oak forest soil, ME	0.64	0. 86	Lucas and Casper, 2008
Black spruce forest soil, AK	0.82		Allison et al., 2008
Grassland, MN			Keeler et al., in press
Litter		1.67	
Soil		1.09	
Aspen forest, MN			Keeler et al., in press
Litter		0.97	
Soil		1.03	
Maple forest, MN			Keeler et al., in press
Litter		0.55	
Soil		0.98	
Oak forest, MN			Keeler et al., in press
Litter		0.89	
Soil		0.88	
White pine forest, MN			Keeler et al., in press

Table 2 (*continued*)

System	POX	PER	Reference
Soil		0.91	
Bermudagrass soil, NC	1.07/0.76/0.83	1.09/1.00/1.03	Lyyemperumal and Shi, 2008
Tall fescue soil, NC	1.08/0.88/0.83	0.98/0.95/0.98	Lyyemperumal and Shi, 2008
Annual grassland soil, CA	1.00		Henry et al., 2005
Semiarid grassland soil, NM	1.02	0.98	[3]Zeglin et al., 2007
Tallgrass prairie soil, KN	1.07	3.76	Zeglin et al., 2007
Tallgrass veld soil, SA	4.63/0.33		Zeglin et al., 2007
Semiarid grassland soil, NM			[3]Stursova et al., 2006
Microbiotic crust	1.02	1.02	
Rhizosphere	1.12	1.10	
Corn/rye agriculture soil, KY			Matocha et al., 2004
No till	0.79/0.62		
Plowed	0.94/1.22		

9.1. Nitrogen enrichment

Nitrogen enrichment increases decomposition rates for litter with low lignin and N contents and slows rates for litter with high contents of lignin and other secondary compounds (Fog, 1988; Berg and Matzner, 1997; Knorr et al., 2005). Carreiro et al. (2000) linked these responses to changes in phenol oxidase activities, both positive and negative, a finding that has been confirmed in other decomposition studies (Allison and Vitousek, 2004). From an ecological perspective, it is surprising that N control on expression of phenol oxidase and peroxidase enzymes differs at the microbial community level given the diverse functions these enzymes serve at the organismal level. Even more surprising is that the phenomena extend to the ecosystem scale as well (Table 2).

In soils, chronic N enrichment has been associated with progressive loss of microbial biomass and reduced rates of respiration (Frey et al., 2004; DeForest et al., 2004b; Zeglin et al., 2007; Treseder, 2008). These biomass declines do not appear to be accompanied by major changes in microbial community composition (Gallo et al., 2004; DeForest et al., 2004a; Lucas et al., 2007), although selective losses or shifts in dominance within particular groups like mycorrhizal fungi have been reported (Treseder, 2004; Lucas and Casper, 2008; Porras-Alfaro et al., 2007, 2009; Allison et al., 2008). These responses have generally been accompanied by net declines in phenol oxidase and peroxidase activities (Table 2). In some cases, these oxidative responses have been correlated with increases in soil organic matter concentration (Sinsabaugh et al., 2005; Matocha et al., 2004) and DOC release (Waldrop and Zak, 2006; Sinsabaugh et al., 2004; Pregitzer et al., 2004). Analyses of laccase gene abundance and diversity suggest that loss of soil oxidative enzyme activity is the result of reduced expression, rather than shifts in community composition (Blackwood et al., 2007; Hofmockel et al., 2007; Lauber et al., 2009b; Hassett et al., 2009).

However, most ecosystem studies of N enrichment have been conducted in temperate and boreal forests where N deposition rates are generally greatest and soil microbial communities are dominated by symbiotic and saprotrophic basidiomycetes (Allison et al., 2008; Lauber et al., 2008). Although the number of studies is small, it appears that grassland soils, whose microbial communities are dominated by Glomeromycota and Ascomycota (Porras-Alfaro et al., 2009), do not show depressed oxidative activity, or increased SOM content, in response to N (Henry et al., 2005; Zeglin et al., 2007; Lyyemperumal and Shi, 2008; Table 2, Fig. 3). This distinction is consistent with evidence that ectomycorrhizal basidiomycetes express a mix of phenol oxidases, proteases and chitinases for the purpose of mining nitrogen from humus, rather than for carbon acquisition (Talbot et al., 2008; Courty et al., 2009).

In northern temperate forests, ectomycorrhizal fungi account for about half of basidiomycete laccase gene diversity (see metagenomics section). The loss of phenol oxidase activity in these ecosystems with no decline in laccase gene diversity suggests that ectomycorrhizal fungi may reduce phenol oxidase expression when nitrogen availability is high. This loss of activity may increase the concentration of refractory carbon and inhibitory phenols, creating a negative feedback on microbial biomass and respiration.

Nearly all efforts to understand microbial responses to N enrichment have focused on fungi, but declines in phenol oxidase activity may also have a bacterial component. Sinsabaugh et al. (2004) conducted bioassays in which bacteria were reciprocally inoculated into dissolved organic matter extracted from control and N amended soils; phenol oxidase activity was significantly lower in extracts from N amended soils.

The findings of ecosystem studies contrast sharply with results of culture studies described earlier. A few white rot basidomycetes reduce peroxidase expression in response to N enrichment, but many do not and laccase expression in general does not appear to be N regulated (Rabinovich et al., 2004; Allison et al., 2009); however, as noted above that may not be true for ectomycorrhizal basidiomycetes. But in the field, strong and divergent responses are observed for decomposer communities associated with labile vs. recalcitrant material even within the same system. Data from Allison and Vitousek (2004) and Sinsabaugh et al. (2002) suggest that a critical LCI value of 0.4 separates litter types that show net positive or neutral phenol oxidase responses to N enrichment from those that show net negative responses (Fig. 4). Metaproteomic studies that match environmental enzymes to specific producers

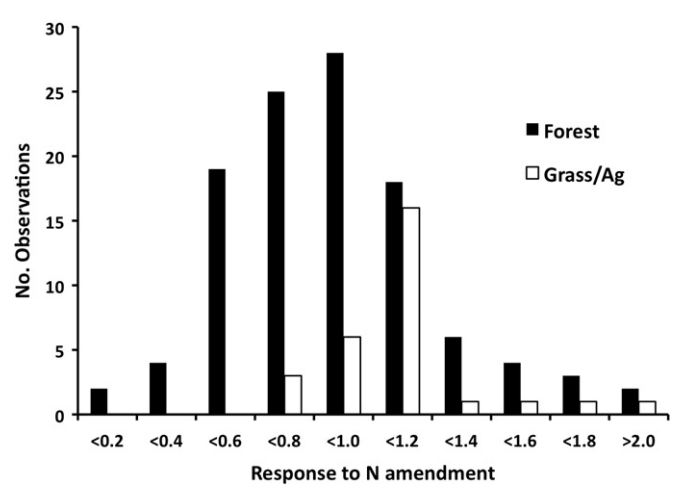

Fig. 3. Summary of phenol oxidase responses to experimental nitrogen enrichment by ecosystem type. Effects are expressed as a response ratio (R, treatment/control). The data are taken from Table 2. For temperate and boreal forest ecosystems, phenol oxidase activity tends to decline with N enrichment (median $R = 0.85$). This response is less common in grassland and agricultural ecosystems (median $R = 1.0$).

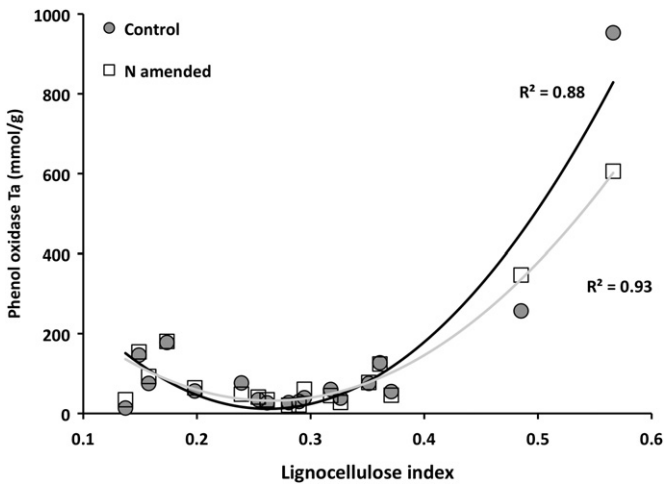

Fig. 4. Phenol oxidase turnover activity (Ta, mmol/g) of decomposing leaf litter in control and nitrogen amended treatments in relation to initial lignocellulose index (LCI, lignin/(lignin + cellulose)). The regressions highlight findings that N addition tends to depress phenol oxidase activity in recalcitrant (high LCI) litter. Data are taken from Sinsabaugh et al. (2002) and Allison and Vitousek (2004). Second order regression for control treatment: $8864(LCI)^2-4654(LCI)+623$. Second order regression for N amended treatment: $6303(LCI)^2-3347(LCI)+476$.

may resolve these disparate observations, but it seems clear that N enrichment generates positive and negative interactions within microbial communities that are not predicted by culture studies.

9.2. Carbon dioxide enrichment

A few studies have measured phenol oxidase and peroxidase activities in experiments that manipulate atmospheric CO_2 at the ecosystem scale. The effect of atmospheric CO_2 enrichment on soils is mediated by plant community responses including changes in net primary production, litter production, belowground carbon allocation and associated changes in composition of litter or exudates. Larson et al. (2002) found no change in soil phenol oxidase or peroxidase activities with elevated CO_2 and elevated $CO_2 + O_3$ treatments for an aspen (*Populus tremuloides*), birch (*Betula papyrifera*), sugar maple (*Acer saccharum*) system. Sinsabaugh et al. (2003) observed no effects of CO_2 enrichment in a sweetgum (*Liquidamber styraciflua*) system. Likewise, no effects were found for an annual grassland (Henry et al., 2005), but phenol oxidase activity did increase by 20% across treatments with water addition. In contrast, Finzi et al. (2006) reported significant declines in phenol oxidase activity (litter R 0.54, soil R 0.76) in a loblolly pine (*Pinus taeda*) system subject to elevated CO_2 over a five year period. Peroxidase activities showed no response. The lower phenol oxidase activity was attributed to an increase in the availability of labile carbon as a result of CO_2 stimulated increases in plant growth.

9.3. Land management

Land management impacts microbial community composition and activity through changes in organic matter and nutrient inputs and disturbance frequency. Lauber et al. (2008) analyzed soil microbial communities in relation to land use in the southeastern US. They reported that edaphic variables rather than land use per se was the best predictor of microbial community composition. For bacteria, community composition was most closely tied to soil pH, supporting broader analyses by Fierer and Jackson (2006) and Lauber et al. (2009a). For fungi, community composition was tied to soil nutrient status, in particular extractable P and C:N ratio (Lauber et al., 2008). In this context, phenol oxidase and peroxidase activities in soil should vary with land use in relation to the magnitude of their edaphic responses to disturbance.

9.3.1. Fire

Fire is frequently used as a management tool in forest and rangeland. Prescribed burns remove labile carbon and nitrogen, reset plant succession and create recalcitrant pyrolysis products. The effects on soil processes depend on intensity and frequency. Boerner and Brinkman (2003) found soil phenol oxidase activity increased 20–80% in oak forest plots subjected to annual or biannual prescribed burns to remove understory vegetation. In contrast, β-glucosidase and phosphatase activities were reduced by 5–50%. However, studies following single prescribed fire events in the same region found that phenol oxidase activity in soils was similar to that of control sites (Boerner et al., 2000, 2005). Collectively, these studies suggest that frequent burns reduce labile organic matter and increase recalcitrant fractions.

In both southern oak and loblolly pine forests (*P. taeda*), thinning was shown to increase phenol oxidase activity in soil relative to prescribed fire treatment (Boerner et al., 2006; Giai and Boerner, 2007), presumably because of greater organic matter availability. A continental scale comparison of the effects of prescribed burns on soil EEA showed that phenol oxidase activity declined by an average of 15% relative to unburned controls while mechanically thinned sites showed no change (Boerner et al., 2008).

Waldrop and Harden (2008) studied the effects of wildfire on black spruce (*Picea mariana*) forests in Alaska. Five years after the event, the O horizon had significantly less microbial biomass and lower phenol oxidase activity than unburned control sites with larger effects at sites not underlain by permafrost (phenol oxidase R 0.51 at permafrost sites, 0.38 at non-permafrost sites). Phenol oxidase and microbial biomass in A horizons were not affected. Across sites, phenol oxidase activity was positively correlated with fungal biomass and the decomposition of ^{13}C-lignin. In contrast, Zhang et al. (2005) found that phosphatase, phenol oxidase and peroxidase activities increased in spruce forest (*Picea balfouriana*) soils following fire, even though organic matter content decreased.

Artz et al. (2009) studied Eucalyptus dominated forest in Australia subject to long term fire management. Sites with burn frequency of 2 and 4 years had a third and a half of the phenol oxidase activity in the top 10 cm of soil compared to control. Fungal biomass was not affected by treatment, but the diversity of basidiomycete laccase genes was greater in the burned treatment.

White and Stursova (unpubl.) measured chlorophyll and EEA in the biological crusts of an arid grassland five years after a prescribed burn (Sevilleta, NM, USA). Compared to unburned controls, the biological crusts had lower concentrations of chlorophyll (R 0.40) but similar hydrolytic activities. But phenol oxidase and peroxidase activities were 5X greater in the burned area, presumably reflecting lower availability of labile carbon.

9.3.2. Agriculture

Conversion of native ecosystems to agriculture typically leads to loss of soil organic matter, particularly labile organic matter, which in general should increase phenol oxidase activity in soil, particularly on an organic matter specific basis. Waldrop et al. (2000) reported that pineapple plantations in Tahiti had 50–55% less soil carbon and nitrogen and 75% less microbial biomass than native tropical forest. These changes were accompanied by 10X and 30X increases in phenol oxidase activity per g OM. In contrast, Monkiedje et al. (2006) compared five cropping systems on former forest land in Cameroon found that declines in microbial biomass and phenol oxidase activity with intensive agriculture. In the temperate southeastern US, nitrogen fertilization was shown to

depress phenol oxidase activity in no till corn/rye systems (Matocha et al., 2004). Tilled systems had lower phenol oxidase activity and SOM but activity was not affected by N amendment. Rezacova et al. (2007) reported that both organic and mineral fertilization treatments increased peroxidase activities in agricultural soils with concomitant decreases in fulvic acid aromaticity and humic acid molecular size. Similarly, Zibilske and Bradford (2007) found a negative correlation between extractable polyphenols and phenol oxidase and peroxidase activities in decomposing cowpea and sorghum crop residues.

Benitez et al. (2006) compared olive orchard management practices in Spain. Soil phenol oxidase activity was lowest under a no till, no herbicide regime. Waldrop et al. (2003) compared post-harvest practices at logged Ponderosa pine (*P. ponderosa*) stands in California. Compared to control sites, four post-harvest practices reduced forest floor phenol oxidase activities by 50–75%. A litterbag study found that phenol oxidase activity in decomposing pine foliage was reduced by 30–60% corresponding with declining mass loss rates.

Exotic species invasion may also affect soil phenol oxidase activity. Li et al. (2006) found that SOM beneath a subtropical broadleaf evergreen forest declined progressively with invasion by an exotic understory plant (*Mikania micrathra*) while phenol oxidase activity progressively increased (R 1.3–2.8).

9.4. Peatland

Mid to high latitude heathlands and peatlands have been a particular focus for studies of phenol oxidase and peroxidase activities, because their large carbon stores have the potential to generate positive feedback on atmospheric greenhouse gas accumulation. Peat accumulations are the product of an environment characterized by low oxygen availability (result of water logging), low pH and low temperature; conditions which limit decomposition to the extent that these stores are effectively isolated from the global carbon cycle. Most peatland studies attempt to evaluate the effects of various warming and drying scenarios, the predicted effects of global climate change at high latitude, on microbial activity and trace gas emission. The high phenolic content of peat is inhibitory to microorganisms and extracellular enzymes. Thus phenol oxidase and peroxidase activities are especially critical to understanding potential responses to global climate change (Freeman et al., 1996, 2001, 2004).

Fenner et al. (2005a) using microcosms reported that simulated drought increased the diversity and abundance of phenol-degrading bacteria, respiration, and phenol oxidase and β-glucosidase activities, and decreased the concentration of phenolics. Simulated rainfall produced the opposite effects. In contrast, Toberman et al. (2008a) reported a decline in fungal diversity and phenol oxidase activity during summer drought in a manipulated heathland in Wales. In a related study, Toberman et al. (2008b) conducted drying and wetting manipulations to identify the optimal moisture content of peat for phenol oxidase activity.

Williams et al. (2000) studied phenol oxidase activity in *Sphagnum* and *Carex* wetlands in New York. Across systems activity increased exponentially with solution pH. They concluded that phenol oxidase activity in *Sphagnum* wetland was limited more by pH than by aeration. Activity was positively related to phenolic concentration in *Carex* wetland but not *Sphagnum*, which had much greater concentrations. The results suggest that phenolics may induce phenol oxidase at low concentration but inhibit activity at high concentration.

Like drought effects, the relationship between phenol oxidase activity and phenolic concentration has generated conflicting results. Fenner et al. (2005b) found that phenol oxidase activity was positively correlated with phenolic concentration across seasons. Fenner et al. (2007) reported that CO_2 and warming increased DOC and phenolic export from peat but depressed phenol oxidase and β-glucosidase activities by 58% and 27%. These effects were accompanied by a large increase in root growth.

Collectively, these studies establish that the interactive effects of moisture, oxygen, temperature and pH on phenol oxidase activity and, by extension, the mineralization and export of carbon from peatland are complex and sensitive to alterations in environmental conditions (Laiho, 2006). The results also reinforce the point that the controls on phenol oxidase activity are distinct from other microbial activities with the potential to generate both positive and negative feedbacks on ecosystem processes.

10. Soil organic matter storage

At the ecosystem scale, phenol oxidase and peroxidase activities often correlate with mass loss rate in litter and particulate organic matter, and with changes in SOM content in response to experimental manipulations. At one extreme, peatlands appear to be enzyme-limited systems where low phenol oxidase activities contribute to organic matter accumulation (Freeman et al., 2001, 2004). At the other extreme, arid ecosystems appear to be substrate-limited systems where high phenol oxidase and peroxidase potentials are thought to limit SOM content (Henry et al., 2005; Collins et al., 2008; also see Nadeau et al. (2007) for role of hydrolytic enzymes). But unlike hydrolase activities, there is no correlation between these oxidative activities and SOM content on a global scale: for LN(phenol oxidase, nmol h^{-1} g^{-1}), $r = 0.20$, $p = 0.11$, $n = 63$; for LN(peroxidase), $r = 0.13$, $p = 0.39$, $n = 43$. Only soil pH has a univariate statistical link to potential oxidative activity and that accounts for only a third of the variance (Fig. 1). A multiple linear regression model that includes soil pH, mean annual temperature (MAT) and mean annual precipitation (MAP) along with potential phenol oxidase activity (as LN (activity/g soil)) describes only 36% of the variance in SOM content across ecosystems ($F = 8.2$, $p < 0.0001$, $n = 63$, pH $p = 0.02$, MAT $p = 0.004$, MAP $p = 0.003$, POX $p = 0.006$). A similar model for peroxidase activity describes 32% of the variance ($F = 6.1$, $p = 0.002$, $n = 43$, MAT $p = 0.004$, MAP $p = 0.002$, PER $p = 0.06$).

Factors that attenuate the relationship between oxidative enzyme activity and SOM are easy to identify but difficult to model. One factor is that phenol oxidases and peroxidases have multiple functions on both the organismal and ecosystem scales (Fig. 5). On the organismal scale, the abundance and functional distribution of

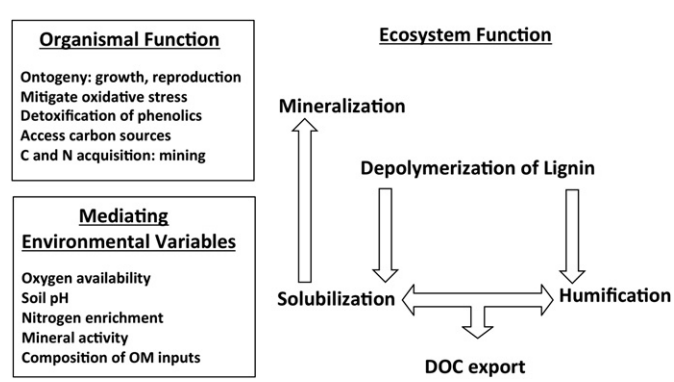

Fig. 5. Plants and microorganisms express intra- and extracellular phenol oxidase and peroxidase enzymes for a variety of purposes. Once released into the environment, activity and turnover are affected by multiple interacting variables. At the ecosystem scale, the aggregate activity of these enzymes mediate several processes that control the concentration and composition of soil organic matter.

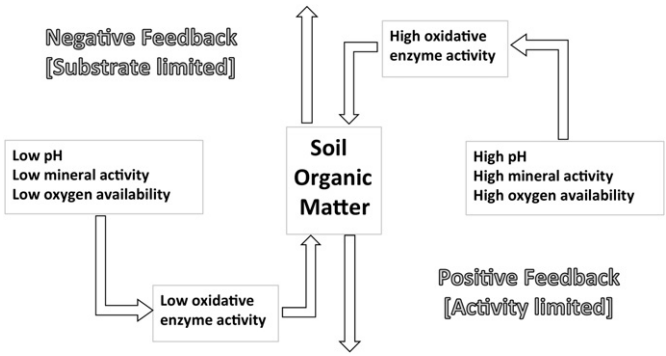

Fig. 6. Environmental conditions that promote in situ oxidative enzyme activity in soil drive a substrate-limited feedback cycle that keeps soil organic matter concentration low. Conditions that restrict in situ oxidative activity sustain an activity-limited feedback cycle that increases stores of soil organic matter.

phenol oxidases and peroxidases will vary with plant and microbial community composition. At the ecosystem scale, SOM is the net product of several processes mediated by oxidative activity that are difficult to partition and likely to vary in relation to critical environmental variables, particularly water and oxygen availability, and the composition of organic matter inputs (Fig. 5). Another factor is sampling error. Given the large spatiotemporal variation in phenol oxidase and peroxidase activities, it is likely that the extent of sampling in most studies does not provide a good estimate of mean annual activity.

Analysis of the residuals from the phenol oxidase multiple regression model described above shows that the range of deviations is greatest for SOM contents of 10–15% and steadily narrows at both higher and lower values. The magnitude of deviations declines with increasing soil pH ($r = -0.25$) and increasing mean annual temperature ($r = -0.13$), and increases with increasing mean annual precipitation ($r = 0.32$). A multiple correlation with all three variables has an r coefficient of 0.52 ($p = 0.0003$). In general, SOM concentration tends to be underestimated in soils that are either cold, wet, low pH or some combination of these and underestimated in systems with opposing characteristics. This pattern suggests that phenol oxidase and peroxidase activities can contribute to either positive or negative feedback cycles under varying suites of environmental variables (Fig. 6), as the studies from peatlands and arid grasslands indicate.

Positive feedback cycles are the product of a substrate-limited system, i.e. edaphic and climatic conditions are such that potential activities are sufficient to degrade organic matter at rates comparable to inputs. Arid systems may be disposed to positive feedback because drought increases oxidative stress which induces phenol oxidase expression. Once released into the environment, enzyme stabilization on mineral surfaces and high pH sustain high activity potentials. In contrast, negative feedback cycles define an activity-limited system in which environmental conditions reduce enzyme function to the extent that organic matter degradation rates fall below input rates. Disruption of any of the three catalytic processes (lignin depolymerization, humification and phenol degradation) that control SOM storage can create a negative feedback. Ecosystem manipulations show that changes in climate, ground cover, and nitrogen input can directly or indirectly affect phenol oxidase and peroxidase activities in situ, initiating a shift to a new system state. In some cases, these ecosystem responses can be modeled as functions of soil pH, temperature or moisture availability. But at larger scale, the magnitude and direction of responses vary with microbial (and plant) community composition, and limited knowledge of the origin and regulation of these enzymes in relation to microbial diversity impedes development of a predictive framework.

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