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RESEARCH ARTICLE

A comparison of lignin-degrading enzyme activities in forest floor layers across a global climatic gradient

Kazumichi Fujii[1,*], Yuji Nakada[2], Kiwamu Umezawa[3], Makoto Yoshida[2], Makoto Shibata[4], Chie Hayakawa[5], Yoshiyuki Inagaki[1], Takashi Kosaki[6], Ryan Hangs[7]

1 Forestry and Forest Products Research Institute, Tsukuba 305-8687, Japan
2 Institute of Agriculture, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan
3 Faculty of Agriculture, Kindai University, Nara 631-8505, Japan
4 Niigata Agro-Food University, Niigata 959-2702, Japan
5 Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan
6 Faculty of International Communication, Aichi University, Nagoya 453-8777, Japan
7 Department of Soil Science, Saskatchewan University, Saskatchewan S7N 5A8, Canada

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ABSTRACT

Rapid litter turnover in tropical forests and during summer seasons might be due to increases in ligninolytic enzyme activities during warmer periods. We compared ligninolytic enzyme activity [lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac)] in the organic layers of forest soils across a global climate gradient. As expected, MnP activities in fresh litter layers increased with increasing air temperature. Litter Mn/lignin ratios correlate positively with MnP activity and more rapid litter turnover in warmer climates. In contrast, LiP and Lac activities are regulated by site-specific conditions. Lac activity is commonly observed in less acidic fresh litter layers, while LiP activity localizes in acidified and lignin-rich deeper organic layers. The widespread occurrence of MnP and an increase in MnP activities in warmer climates support efficient lignin degradation in the tropics and during summer seasons. High Mn/lignin ratios in fresh litter could be an indicator of lignin degradability by MnP-producing fungi across global climate gradients.

1 Introduction

Lignin degradation is a rate-limiting step in litter decomposition in forest ecosystems (Berg et al., 2007; Osono, 2007). Lignin, as well as cellulose and hemicelluloses, is a major constituent of plants. The complex aromatic macromolecular structure of lignin differs from cellulose and is resistant to microbial attack (Kirk, 1984). Also, lignin degradation requires oxidative reactions by a complex set of ligninolytic enzymes (Ten Have and Teunissen, 2001). Despite chemical and biological recalcitrance, rapid litter turnover in warmer temperate and tropical forests is supported by efficient lignin degradation. Understanding of enzymes involved in lignin oxidation in the forest soils and under laboratory conditions has gradually improved (Kellner et al., 2014). However, the linkage between ligninolytic enzyme activity and litter turnover remains unclear at global and local scales.

White-rot basidiomycete fungi decompose lignin effectively by secreting enzymes (Hofrichter, 2002). Lignin (LiP) and manganese peroxidases (MnP) are efficient ligninolytic enzymes (Leonowics et al., 2001), and a variety of the other ligninolytic enzymes [e.g., laccase (Lac), versatile peroxidase, aromatic peroxidase, and chloroperoxidase] are also reported (Wong, 2009). The presence of MnP has been confirmed in

* Corresponding author
E-mail address: fjkazumichi@gmail.com (K. Fujii)

organic layers, and woody debris in some temperate forests (Yoshida et al., 2011; Kellner et al., 2014). Still, patterns of ligninolytic enzyme activity remain unreported among forests that differ in climate, vegetation, and soil type.

Climate, vegetation, and soil types likely affect the activity of LiP, MnP, and Lac in response to soil properties (pH, Mn levels), and microbial communities, lignin, Mn, and nitrogen (N) concentration in fresh litter (Criquet et al., 2000; Osono, 2007). More rapid litter turnover in warmer climates, such as in tropical forests during summer seasons requires increased enzymatic activity for efficient lignin degradation. For example, greater annual precipitation (leaching) leads to a decrease in soil pH (Slessarev et al., 2016). Lignin degradation by specific fungi may increase by production of LiP, the ligninolytic enzyme with the lowest pH optimum (Fujii et al., 2013a). Also, MnP activities could be stimulated by high litter Mn levels (Bonnarme and Jeffries, 1990; Berg et al., 2007).

Variation in litter and soil properties may affect dominant ligninolytic enzymes and litter turnover (Tuomela et al., 2002; Berg et al., 2007). Litter Mn may promote litter degradation by stimulating MnP activity. However, relationships between MnP activities and litter turnover has rarely been confirmed under field conditions.

In this study, our objectives are (1) to describe general patterns of ligninolytic enzyme activity across a global climatic gradient, and (2) to examine relations between ligninolytic enzyme activities, litter decomposition rates and chemical and biological properties of the organic layers and soils. Studies targeted parameters such as litter pH, carbon (C)/N ratio, lignin, lignin/N ratio, Mn, Mn/lignin ratio, and fungal activity. We compared ligninolytic enzyme activities from 16 sites, including boreal forests (Canada), temperate forests (Japan, USA), and tropical forests (Indonesia, Cameroon). We also confirmed the identity of ligninolytic enzymes and their sources at a molecular level and analyzed their importance for forest litter turnover using our data along with data sets from previous works.

2 Materials and methods

2.1 Sampling of organic layers

Organic layer samples were collected from 16 upland sites from August 2008 in Canada and in August 2011 in Japan, USA, Indonesia, and Cameroon (Table 1). The fresh litter and humified layers, if any, were collected separately from each location in three replicates. Litter (L), fermented (F), humified (H), and a mixture of F and H layers FH (FH) layers collected correspond to Oi, Oe, Oa, and Oea horizons in soil taxonomy, respectively (Soil survey staff, 2014). Samples were collected from Canada (Sites 1–5), USA (Site 6), Japan (Sites 7–11), Indonesia (Sites 12, 13, 14), and Cameroon (Sites 15, 16) (Table 1). Sites were selected to reflect the variability in lignin and soil pH in boreal, temperate, tropical forests. Boreal forests included aspen forests on less acidic alfisols and coniferous forests on acidic inceptisol and spodosol. Temperate forests included beech forests on less acidic Andisol, beech or oak forests on acidic inceptisol and spodosol, and coniferous forests on the Andisol. Tropical forests included non-N-fixing and N-fixing tree species that differ in lignin and N concentrations and two soil types, ultisol and oxisol, that differ in pH (Fujii et al., 2018).

2.2 Chemical properties of organic horizon samples

Litterfall was collected from three forest plots (20 m $\times$ 20 m) per site using circular litter traps (60 cm diameter) in five replicates. The organic layers were collected from three 30 cm $\times$ 30 cm quadrants per site. Litterfall and organic layer samples were oven-dried at 70°C for 48 h, weighed, and milled. pH values of organic layers (L and FH, or L, F, and H, if any) were measured using a milled litter-to-solution (water) ratio of 1:20 (w/v). Klason lignin concentrations in fresh litter and FH layer samples were determined by digestion with sulfuric acid (Allen et al., 1974). Mn concentrations in fresh litter and FH layer samples were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, SPS1500, Seiko Instruments Inc.) after nitric-sulfuric acid wet digestion. The C and N concentrations were determined using a CN analyzer (Vario Max CN, Elementar Analysensystem GmbH). Organic layer C was assumed to reach steady-state, and decomposition rate factors (yr^{-1}) of C in the organic layer (L + FH) was calculated by dividing litterfall C input (Mg C ha^{-1} yr^{-1}) by organic layer C (Mg C ha^{-1}) (Olson, 1963). These litter degradation rate factors are not precise compared to factors measured using *in situ* litterbag tests, yet we confirmed that litter decomposition rate factors (1.6) are comparable to factors obtained using litter bag tests in a tropical forest (1.5–2.0; Fujii and Hayakawa, 2020). Lignin (%)/N (%) ratios were calculated and used as an indicator of litter recalcitrance (Aerts, 1997). We also calculated Mn per lignin [Mn (%)/lignin (%) ratios] and defined results as indicators of litter Mn availability for ligninolysis.

2.3 Chemical and biological properties of surface soil samples

Soil samples (0–5 cm; A horizon) under the organic layers were air-dried and crushed to pass through a 2-mm sieve before analysis. Soil pH was measured using soil to solution (water or 1 M KCl) ratios of 1:5 after shaking for 1 h. Soil C concentrations were determined using a CN analyzer. Clay content was measured using a pipette method.

To approximate fungal activity, the relative contribution of fungi to glucose-induced respiration in soil (0–5 cm depth) was measured by selective inhibition (Anderson and Domsch, 1973; Joergensen and Wichern, 2008). Field-moist soil samples were amended with ^{14}C-labeled glucose with and without cycloheximide (fungal respiratory inhibitor) (equivalent to 8–24 mg cycloheximide g^{-1} soil, respectively) and incubated at 22°C for 24 h. ^{14}C-CO_2 was collected in 1M NaOH solution and measured using liquid scintillation counting (Aloka, LSC-3050). The percent contribution of fungi to

Table 1 Site description and characteristics of the surface soils.

Site	Location	Vegetation	Cordinates	Annual mean air temperature (°C)	Annual precipitation (mm)	Soil (Soil taxonomy)	Soil pH		Soil C (mg C kg^{-1})	Clay (%)	Fungal respiratory activity (%)
							Water	KCl			
Boreal forest											
1	Saskat-chewan, Canada	Aspen (*Populus tremuloides*)	N52°52', W107°20' (Emma lake)	0.9	424	Alfisols	6.6	5.9	81	14	23
2		Aspen	N53°33', W105°53' (Rosthern)	2.2	347	Alfisols	6.2	5.5	3	19	35
3		Black spruce (*Picea mariana*)	N54°48', W104°48' (La Ronge)	−0.1	484	Inceptisols	4.6	4.0	2	7	55
4		Jack pine (*Pinus banksiana*)	N54°02', W105°54' (Kenderdine)	0.9	424	Alfisols	4.3	3.6	12	14	-
5	British Columbia, Canada	Black spruce	N52°52', W118°29' (Jasper)	3.5	594	Spodosols	5.0	4.1	8	6	50
Temperate forest											
6	Virginina, USA	*Quercus* spp.	N37.27, W80.48 (Jefferson national forest)	10.3	1030	Inceptisol	4.0	3.7	-	30	-
7	Iwate, Japan	Beech (*Fagus crenata*)	N40.00, E140.56 (Appi station)	6.1	1800	Inceptisols	4.5	4.2	80	14	46
8		Beech	N39.46, E141.80 (FFPRI station)	10.5	1229	Andosols	5.0	4.3	158	32	33
9	Kyoto, Japan	Beech	N35°1', E135°47' (Miyadzu)	10.7	1490	Spodosols	3.8	3.5	125	49	67
10	Ibaraki, Japan	Cedar (*Cryptomeria japonica*)	N36.04, E140.06 (Mt. Tsukuba)	14.0	1395	Inceptisols	4.6	4.0	88	31	26
11		Cypress (*Chamaecyparis obtusa*)	N36.04, E140.06 (Mt. Tsukuba)	14.0	1395	Inceptisols	4.6	4.0	88	31	26

(*Continued*)

Site	Location	Vegetation	Cordinates	Annual mean air temperature (°C)	Annual precipitation (mm)	Soil (Soil taxonomy)	Soil pH		Soil C (mg C kg^{-1})	Clay (%)	Fungal respiratory activity (%)
							Water	KCl			
Tropical forest											
12	East Kalimantan, Indonesia	Dipterocarp (*Shorea laevis*)	S0°51', E117°06' (Bukit Soeharto)	26.8	2187	Ultisols	4.0	4.0	36	23	82
13		*Harpullia arborea*	S1°51', E116°02' (Kuaro)	26.8	2256	Oxisols	6.3	5.8	73	55	54
14		*Harpullia arborea*	S1°49', E115°59' (Kuaro)	26.8	2256	Ultisols	5.4	5.0	73	79	54
15	Cameroon	Mimosaceae (*Albizia zygia*)	N4.32, E13.15 (Bertoua)	22.9	1531	Oxisols	4.2	3.9	19	55	-
16		Mimosaceae (*Piptadeniastrum africanum*)	N2.52, E11.09 (Yaounde)	23.8	1650	Ultisols	3.5	3.3	30	47	-
17	Var, France	*Quercuss ilex*	-	14.9	694	Alfisols	-	-	-	-	-
18	Kyoto, Japan	*Quercuss serrata*	N35°1', E135°47' (Mt. Yoshida)	15.9	1490	Inceptisols	4.2	3.7	45	47	54
19	East Kalimantan, Indonesia	Dipterocarp	S1°01', E116°52'	26.8	2427	Ultisols	4.2	3.4	36	27	78
20	Chiang-Rai, Thailand	*Lithocarpus* spp.	N19°50', E100°20' (Rakpaendin)	25.0	2084	Ultisols	5.0	4.1	63	70	36

Data sources of sites information are Gower et al. (1997) for Sites 1–3, Nakane et al. (1997) for Sites 3–5, Megonigal et al. (1997) for Site 6, Yasuda et al. (2012) and Fujii et al. (2019) for Sites 7–8, Fujii et al. (2008) for Site 9, Inagaki et al. (2012) for Sites 10–11, Fujii et al. (2011) for Sites 12–14, Shibata et al. (2017) for Sites 15–16, Criquet et al. (2002) for Site 17, and Fujii et al. (2013b) for Sites 18–20.

total microbial respiration was calculated using differences between $^{14}CO_2$ evolution rates of glucose-amended soils with/without inhibitor. All experiments were performed in triplicate.

2.4 Extraction of ligninolytic enzymes

Ligninolytic enzymes were extracted from 80 g of litter samples in 700 mL of a 0.1 M $CaCl_2$ solution with 0.05% Tween 80 and 20 g polyvinyl polypyrrolidone at room temperature for 1 h on a reciprocal shaker. Suspensions were filtered through a double layer of gauze to remove floating debris then centrifuged at 12 000 *g* for 20 min at 4°C. Supernatants were filtered through 3.0 µm and 1.0 µm filters then dialyzed for 48 h at 4°C in 14 kDa molecular mass cut-off cellulose dialysis tubing against frequently exchanged 2 mM bis-tris [bis (2-hydroxyethyl) imino-tris (hydroxymethyl) methane] buffer, pH 6.0. Supernatants of each extract were concentrated in the same cellulose dialysis tubes covered with polyethylene glycol until a final volume of 1/10 to 1/20 of the initial volume was reached. Most litter extract samples were brown; however, the effects of turbid materials on enzyme assays are reported to be small (Archibald, 1992; Criquet et al., 1999).

2.5 Enzyme activity assay

For all ligninolytic enzyme activities assays, reaction times were 5 min at 30°C. The enzymatic activity was measured at optimal pH, 3.0, 4.5, and 5.7 for LiP, MnP, and Lac, respectively. Extracts boiled for 15 min served as controls for the activity of LiP and Lac, and reaction in a mixture without Mn served as a control for MnP activity. One unit of enzyme activity was defined as the amount of enzyme forming 1 µmol of reaction product per min and was expressed as U g^{-1} dry matter (DM) (µmol min^{-1} g^{-1} DM).

The LiP activity was measured using Azure B as a substrate (Archibald, 1992; Arora et al. 2002). The reaction mixture contained 0.5 mL of sodium tartrate buffer (50 mM), 0.5 mL of Azure B (32 µM), 0.5 mL of H_2O_2 (100 µM), and 0.5 mL of enzyme extract. The reaction was initiated by adding H_2O_2. The oxidation rate of Azure B was measured with a UV-VIS spectrophotometer (UV-1200, Shimadzu) at 651 nm based on an extinction coefficient of ϵ = 48 800M^{-1} cm^{-1}.

MnP activity was measured using phenol red as a substrate (Orth et al., 1993; Arora et al. 2002). The reaction mixture contained 2.0 mL of sodium succinate buffer (50 mM), 2.0 mL of sodium lactate (50 mM), 0.8 mL of Mn sulfate (0.1 mM) (or an equimolar amount of EDTA for control), 1.4 mL of phenol red (0.1 mM), 0.8 mL of H_2O_2 (50 µM), 2.0 mL of albumin (0.1%), and 1.0 mL of enzyme extract. The reaction was initiated by adding H_2O_2. A 2 mL aliquot of the reaction mixture was removed, to which 40 µL of 5 M NaOH was added. The oxidation rate of the phenol red was measured at 610 nm based on an extinction coefficient of ϵ = 4 460 M^{-1} cm^{-1}.

Lac activity was measured using syringaldazine as a substrate (Criquet et al., 1999). The reaction mixture contained 2.5 mL of phosphate-citrate buffer (0.1 M), 0.1 mL of syringaldazine (5 mM), and 0.5 mL of enzyme extract. Oxidation rate of syringaldazine to quinone was measured at 525 nm using syringaldazine extinction coefficient of ϵ = 65 000 M^{-1} cm^{-1}.

2.6 Extraction and sequencing of RNA encoding ligninolytic enzymes

Enzyme producers and confirmation of true ligninolytic enzyme activities were examined with transcripts of RNA encoding ligninolytic enzymes. RNA extraction and cDNA synthesis used 100 mg of composite organic layer samples (L, F, and H) at Site 9. Organic material was milled, extracted and purified using RNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. Site 9 was selected due to a wide variation in pH and Mn levels among L, F, and H layers. Approximately 500 ng of purified DNA-free RNA was used as a template for reverse transcription, and the reaction conducted at 50°C using reverse transcriptase, SuperScript® III (Invitrogen).

We used a combination of degenerate primers for class II peroxidase, peroxiF1 (5′ -CGI CTS ACI TTC CAY GAY GC BAT- 3′) and peroxiR1 (5′ -GT IGA GTC RAA SGG IGY ISC-3′), designed using multiple alignments of the known MnPs (Yoshida et al., 2011). PCR amplification used a Thermal Cycler Dice (Takara, Japan) in a 20 µL PCR reaction of 10.0 µL Taq HS Low DNA (TaKaRa), 0.4 µL of 10 µM forward and reverse primer and 0.5 µL cDNA template. Cycling used initial denaturation for 3 min at 94°C, 35 cycles of denaturation (5 s at 94°C), annealing (5 s at 60°C), and elongation (10 s at 68°C), and a final elongation step for 5 min at 72°C. PCR products of expected sizes (MnP: ~400 bp) were gel purified using a Wizard® SV Gel and PCR Clean-Up System (Promega). PCR amplification then used a 20.0 µL PCR reaction of 0.1 µL Taq HS Low DNA (TaKaRa), 2.0 µL of 10 µM forward and reverse primers and 1.0 µL cDNA template. Cycling used in initial denaturation for 3 min at 95°C, 30 cycles of denaturation (10 s at 98°C), annealing (30 s at 60°C), and elongation (30 s at 60°C), and a final elongation step for 5 min at 72°C. PCR products of expected sizes (MnP: ~400 bp) were gel purified using Wizard® SV Gel and PCR Clean-Up System (Promega), and cloned into the pGEM-T easy vector using the pGEM-T easy Cloning Kit (Promega) for Sequencing. Nine positive clones were sequenced at Macrogen Japan and identified with blastx using the GenBank database (National Center for Biotechnology Information, USA National Library of Medicine).

2.7 Calculations and statistics

All results are expressed on an oven-dry weight basis (70°C, 48 h) and are the mean of three replicates. Statistical significance of simple linear regressions was tested using a significance level of 0.05 unless otherwise stated. The statistical analyses were performed with SigmaPlot 12.3 (SPSS Inc., 2013).

3 Results

3.1 Physicochemical properties of organic layers and soil samples

Soil pH (water) was negatively correlated with annual precipitation ($r = -0.44$, $p < 0.10$, $n = 16$; Table 1). The fungal respiratory activity in surface soil (0–5 cm; A horizon) was negatively correlated with soil pH (water) ($r = -0.60$, $p < 0.05$, $n = 16$; Table 1). Organic layer pH (FH or L, if not) was positively correlated with pH (KCl) of the surface mineral soil ($r = 0.60$, $p < 0.05$, $n = 16$; Table 2). The soils overlaid by FH layers were consistently acidic [pH (KCl) < 4.0; Table 2].

The lignin concentrations in fresh litter varied widely from 14% to 43% (Table 2). As expected, C/N and lignin/N ratios were significantly ($p < 0.05$) lower in N-fixing forests of Cameroon (Sites 15, 16) than in the other sites (Sites 1–14). Litter Mn/lignin ratios decreased with depth (L > FH) at Site 7, 9, 12–16, except for Site 8 (Table 2).

3.2 Litter decomposition rate factor

Organic layer C stocks varied widely from 2.0 Mg C ha^{-1} in Cameroon (Sites 15, 16) to 30.9 Mg C ha^{-1} in Japan (Site 9; Table 3). Carbon tended to fall within lower values at higher mean annual air temperature (Table 3). Litter decomposition rate ranged from 0.05 yr^{-1} in Japan (Site 9) to 2.25 yr^{-1} in Cameroon (Sites 15, 16) (Table 3). Despite limitations due differences in sampling periods and an assumption of "steady-state" forest ecosystems, litter decomposition rate correlated positively with mean annual air temperature ($r = 0.80$, $p < 0.05$, $n = 16$; Fig. 1A), negatively with organic layer pH ($r = -0.60$, $p < 0.05$, $n = 16$; Fig. 1B), and negatively with the fresh litter Mn/lignin ratio ($r = -0.44$, $p < 0.10$, $n = 16$; Fig. 1C). No significant correlations between organic layer C and lignin/N ratio or litter C turnover and lignin/N ratio were observed. (Fig. 1D), However, organic layer C tended to fall within higher values at higher lignin/N ratio (Fig. 1D).

3.3 Ligninolytic enzyme activities

Consistent with our first hypothesis, MnP activities in L layers correlated positively with mean air temperature during the sampling month (Fig. 2A). In contrast, no correlations between air temperature and LiP or Lac activities were found (Fig. 2B, C). In contrast to detection of MnP activities in a broad pH range (Fig. 3A), high LiP activity was detected in acidic FH layers (pH > 4.3; Fig. 3B) and where lignin concentrations were greater than 35% (Fig. 4). Expression of LiP activity was limited to litter pH below 6.0 (Fig. 3B): Lac activities were detected in litter layers across a broad pH range (3.5–7.9), as seen in MnP activities (Fig. 3A, C). Lac activity was commonly found in fresh litter layers, regardless of pH (Fig. 3C).

Two boreal aspen forests (Sites 1, 2) showed similar MnP and Lac activities, while two black spruce forests (Sites 4, 5) exhibited LiP activity but not MnP and Lac activity (Table 4). Expression patterns of MnP, LiP, and Lac in temperate forest litter differed even among beech forest sites (Sites 7–9; Table 4). The MnP activity was replaced by LiP in deeper layers at Site 9 (Table 4). MnP activity in tropical forests was detected at all sites (Sites 12–16), while LiP activity was detected only in the dipterocarp forest (Site 12; Table 4). LiP activity increased when litter lignin concentration exceeded 35% (Fig. 4). Still, no correlation between LiP activities and the litter lignin/N ratios or lignin concentration existed (Table 4). MnP activity was not correlated with litter Mn concentration but did correlate with litter Mn/lignin ratio (Fig. 5A) and with fungal respiratory activity (Fig. 5B).

3.4 Detection of peroxidase transcripts in the organic layers

We found three transcripts of manganese peroxidase partial [uncultured fungus] (Accession no. AVI23960) in L, F, and H layers and two transcripts of manganese peroxidase 2 [*Phlebia* sp. MG60] (Accession no. BAP05605) in the F layer in samples from Site 9. We also detected two transcripts similar to manganese peroxidase 1 [*Pluteus cervinus*] (Accession no. KDQ28248), one transcript similar to manganese peroxidase partial [uncultured fungus] (Accession no. AY06829). Both were found in beech forests, and one transcript was similar to lignin peroxidase [*Phanerochaete chrysosporium*] (Accession no. ABT17198) (Table S1). All transcripts found in samples from Site 9 exhibited similarity with peroxidases produced by Polyporales. The distribution of these transcripts was consistent with patterns of enzyme activities in the same samples (Table 4).

4 Discussion

Ligninolytic enzyme activities under field conditions are hypothesized to be regulated primarily by temperature across global climatic gradients, and secondarily by local vegetation and soil types (Aerts, 1997). Increased MnP activity at higher air temperature supports this hypothesis (Fig. 1A). However, LiP and Lac activity vary widely due to the site-specific properties other than temperature, e.g., vegetation and soil conditions (Fig. 2B,C).

4.1 Effects of vegetation on ligninolytic enzyme activity in forest floor layers

Vegetation type influences ligninolytic enzyme activities through effects on litter (substrate) quality and related dominant fungal communities (Berg et al., 2007; Osono, 2007). For example, *Ganoderma lucidum* preferentially produces MnP in aspen woods but not in pine woods (Hatakka, 2001). In our study, vegetation effects are supported by consistent patterns of MnP activity detection in two aspen forest sites (Sites 1, 2), LiP activity detection in two black spruce forest sites (Sites 4, 5), and detection of MnP, LiP, and Lac activities in three beech forest sites (Sites 7–9) (Table 4). These findings can be interpreted as control of enzyme activity and dominant fungal communities by

Table 2 Chemical properties of the organic layers in the boreal, temperate, and tropical forests studied.

Site	Location	Vegetation	Soil pH (KCl)	Layer	Depth (cm)	Litter layer pH	C/N ratio	Lignin (%)	Lignin/N	Mn (mg g^{-1})	Mn/lignin ($\times 10^{-3}$)
1	Saskatchewan, Canada	Aspen	5.9	L	0–3	6.5	70	14	20	0.03	0.2
2	Boreal	Aspen	5.5	L	0–2	5.3	70	14	20	0.02	0.1
3		Jack pine	4.0	L	0–2	3.7	59	33	39	0.03	0.1
				FH	0–2	3.6					
4		Black spruce	3.6	L	2–5	4.6	68	28	38	0.40	1.4
5	British Columbia, Canada	Black spruce	4.1	L	0–5	3.9	68	28	38	0.07	0.2
	Boreal			FH	0–3	4.2					
6	Virginina, USA	*Quercus* spp.	3.7	L	3–10	5.1	56	24	26	0.44	1.9
	Temperate			FH	0–2	4.5					
7	Iwate, Japan Temperate	Beech	4.2	L	2–5	5.8	30	43	26	0.22	0.5
				FH	0–2	5.1	19	29		0.03	0.1
8		Beech	4.3	L	2–5	5.6	29	43	25	0.02	0.1
				FH	0–2	5.1	24	36		0.03	0.1
9	Kyoto, Japan	Beech	3.5	L	2–4	5.4	34	42	29	0.41	1.0
	Temperate			F	0–2	4.3	25	43		0.25	0.6
				H	2–7	4.0	21	43		0.05	0.1
10	Ibaraki, Japan	Cedar	4.0	L	7–15	5.5	55	31	34	0.06	0.2
11		Cypress	4.0	L	0–3	5.1	65	21	27	0.08	0.4
12	East Kalimantan, Indonesia	Dipterocarp	4.0	L	0–1	5.0	41	41	33	0.65	1.6
	Tropical	*(Shorea laevis)*		FH	1–3	4.5	24	43		0.37	0.9
13		*Harpullia arborea*	5.8	L	0–2	6.5	38	35	27	0.16	0.5
14		*Harpullia arborea*	5.0	L	0–2	7.0	32	27	17	0.65	2.4
15	Cameroon	Mimosaceae	3.9	L	0–1	7.1	17	24	8	0.77	3.2
	Tropical	*(Albizia zygia)*		FH	1–2						
16		Mimosaceae *(Piptadeniastrum)*	3.3	L	0–1	5.4	22	31	14	1.06	3.4
				FH	1–3						
Literatures											
17	France	*Quercuss ilex*	-	L	-	5.8	46	15	14	0.6	4.0
18	Japan	*Quercus serrata*	3.7	L	0–2	5.4	34	40	27	1.90	4.8
				FH	2–5	5.0					
19	Indonesia	*Quercus serrata*	3.4	L	0–1	5.0	41	46	37	0.63	1.4
				FH	1–2	4.6					
20	Thailand	*Lithocarpus*	4.1	L	0–1	5.9	47	24	23	0.64	2.7

Data sources of C/N ratio are Gower et al. (1997) for Sites 1–3, Nakane et al. (1997) for Sites 3–5, Megonigal et al. (1997) for Site 6, Yasuda et al. (2012) and Fujii et al. (2019) for Sites 7–8, Fujii et al. (2008) for Site 9, Inagaki et al. (2012) for Sites 10–11, Fujii et al. (2011) for Sites 12–14, Shibata et al. (2017) for Sites 15–16, Fioretto et al. (2007), Maisto et al. (2013), and Criquet et al. (2000) for Site 17, and Fujii et al. (2013b) for Sites 18–20. L (litter), F (fermented), H (humified), and FH (mixture of F and H layers) corresponds to Oi, Oe, Oa, and Oea horizons in soil taxonomy, respectively (Soil survey staff, 2014).

Table 3 Litterfall, organic layer carbon stock, and decomposition rate factor in the boreal, temperate, and tropical forests studied.

Site	Location	Vegetation	Litterfall-C (Mg C ha^{-1} yr^{-1})	Organic layer C stock (Mg C ha^{-1})	Decomposition rate factor (yr^{-1})
1	Saskatchewan, Canada (Boreal)	Aspen	1.2	7.0	0.17
2		Aspen	1.2	6.5	0.18
3		Jack pine	0.5	10.5	0.05
4		Black spruce	1.3	5.4	0.25
5	British Columbia, Canada (Boreal)	Black spruce	1.3	24.5	0.05
6	Virginina, USA (Temperate)	*Quercus* spp.	3.0	5.1	0.59
7	Iwate, Japan (Temperate)	Beech	1.6	8.4	0.19
8		Beech	1.8	8.0	0.22
9	Kyoto, Japan (Temperate)	Beech	2.1	30.6	0.07
10	Ibaraki, Japan	Cedar	3.6	7.2	0.50
11		Cypress	3.1	4.1	0.76
12	East Kalimantan, Indonesia (Tropical)	Dipterocarp	4.1	3.5	1.17
13		*Harpullia arborea*	4.8	4.1	1.17
14		*Harpullia arborea*	4.0	2.9	1.38
15	Cameroon (Tropical)	Mimosaceae	4.5	2.0	2.25
16		Mimosaceae	4.5	2.0	2.25
Literatures					
17	France (Temperate)	*Quercuss ilex*	-	-	-
18	Japan (Temperate)	*Quercuss serrata*	2.9	3.4	0.85
19	East Kalimantan, Indonesia (Tropical)	Dipterocarp	3.6	4.5	0.80
20	Thailand (Tropical)	*Lithocarpus* spp.	4.0	2.6	1.54

Data sources of litterfall, organic layer C stock, and C/N ratio are Gower et al. (1997) for Sites 1–3, Nakane et al. (1997) for Sites 3–5, Megonigal et al. (1997) for Site 6, Yasuda et al. (2012) and Fujii et al. (2019) for Sites 7–8, Fujii et al. (2008) for Site 9, Inagaki et al. (2012) for Sites 10–11, Fujii et al. (2011) for Sites 12–14, and Shibata et al. (2017) for Sites 15–16.

common traits shared by litterfall and woody materials, e.g., lower lignin and higher pH of aspen litter and woody debris.

Fungal enzyme production is triggered by N starvation (Kirk and Farrell, 1987), and LiP production may be limited in litter layers with low C/N and lignin/N ratios. Lack of LiP activity in N-fixing Mimosaceae forests (Sites 15, 16; Table 4) is consistent with this hypothesis. However, LiP detection over a broad range of lignin/N and C/N ratio (Table 4) suggests that lignin/N or C/N ratio may not be a sensitive indicator of N starvation that triggers fungal LiP production. Further, high LiP activity at lignin/N ratios > 25 and at lignin concentration > 35% (Fig. 4) suggest that high lignin concentration is a prerequisite for expression of LiP activity. Low pH and LiP producers in the deeper organic layers are additional requirements (Fig. 3B). This suggestion is consistent with the finding that LiP evolved in acidic conditions favorable for oxidation of recalcitrant non-phenolic structures of lignin (Oyadomari et al., 2003).

The MnP activity is common at all sites except, for black spruce forests (Table 4) sites, MnP activities in temperate and tropical forests are comparable to activity reported for a Mediterranean oak forest (Criquet et al., 1999; Table 4). Further, transcripts encoding MnP in a Japanese beech forest are similar to those found in a Belgium beech forest (AVI23960; Kellner et al., 2014) and transcripts from white-rot fungi *Phlebia* sp. in Japan (BAG12561; Kamei et al., 2008) (Table S1). We cannot exclude all possibilities that the ligninolytic enzyme activities encompass pseudo-positive reactions. However, the occurrence of enzymes and efficient producers (white-rot basidiomycete fungi or Polyporales) are supported by detection of several transcripts of RNA that encodes ligninolytic enzymes (Table S1). This result partly explains why MnP activity is increased under favorable conditions for fungal growth (Fig. 5B). The positive correlation between MnP activity and litter Mn/lignin ratio (Fig. 5A) suggests an important link between Mn and expression of MnP activity (Berg et al., 2007). Mn^{2+} is oxidized to Mn^{3+} in lignin oxidation by MnP. The complex of Mn^{3+} - di-carboxylic organic acids (e.g., oxalate, malate, and malonate) attacks the phenol structure of lignin as diffusible oxidant (Hatakka, 2001). An increase in litter decomposition related to Mn/lignin ratio (Fig. 1C) also supports the hypothesis that high Mn availability could stimulate litter decomposition through increased microbial MnP activity (Berg et al., 2007). Note that the wide variation in MnP activity associated with Mn/lignin ratios implies that MnP/lignin ratios and Mn availability are conditions that stimulate MnP activity. Such stimulation is highly dependent on of MnP producers (fungi), consistent with the positive correlation between MnP activity and fungal

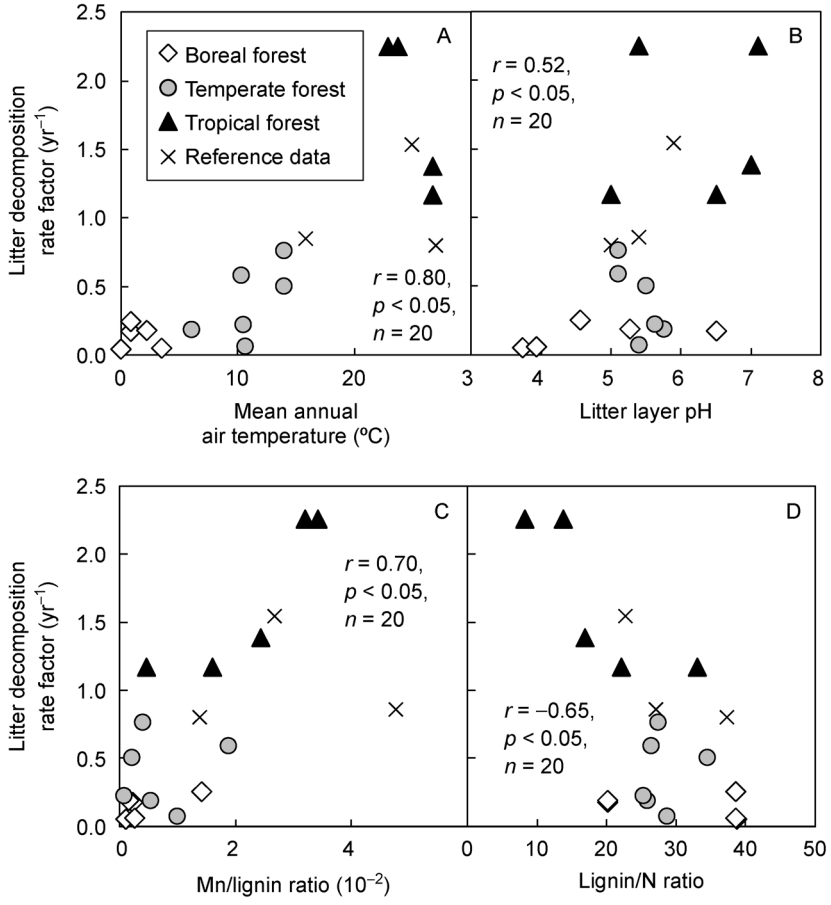

Fig. 1 Litter decomposition rates related to mean annual air temperature (A), litter layer pH (B), fresh litter Mn/lignin ratio (C), and fresh litter lignin/N ratio (D). Reference data are from Criquet et al. (2000) and Fujii et al. (2013b).

respiratory activity (Fig. 5B). Litter Mn/lignin effects, as well as warm climate (Fig. 1A), promote rapid litter turnover in tropical forests (Fig. 1C).

4.2 Effects of soil pH on ligninolytic enzyme activity in organic layers

Soil pH influences ligninolytic enzyme activity directly through its impact on organic layer pH and indirectly by alteration of litter quality and fungal communities (Fujii and Hayakawa, 2013; Tables 1 and 2). Low pH retards microbial and faunal litter decomposition and leads to accumulation of acidic humified layers poor in Mn (Fig. 1B; Staaf, 1987). We hypothesize that production and activity of enzymes are maximized in a pH range close to their optimal pH values (Fujii et al., 2013a), 2.5–3.0 for LiP, 4.5–5.0 for MnP, and 3.0–5.7 for Lac in pure culture systems (Tien and Kirk, 1983; Bollag and Leonowicz, 1984; Glenn and Gold, 1985; Rüttimann-Johnson et al., 1994). Patterns of LiP, MnP, and Lac activity follows reported optimal pH ranges, although a gap exists between pH ranges for field activity and optimal pH obtained from pure culture (Fig. 3). The co-occurrence of LiP, MnP, and Lac is not common (Table 4). This finding can be explained by differences in optimal pH ranges (Fig. 3) and competition among enzyme producers (Rothschild et al., 1999). MnP displays a relatively wide optimal pH range (Fig. 5A) and its activity increases with the activity of broad range of fungal species (Fig. 5B).

Rapid litter turnover without development of an acidified FH layer (Fig. 2B) contributes to favorable (less acidic) pH conditions for expression of MnP and Lac activity in L layers (Fig. 3A, C), consistent with greater microbial and enzyme activities in upper layers (Šnajdr et al., 2008). Compared to LiP, a broader range of basidiomycete fungi can produce MnP. Lac is also produced by the broader fungal community including non-basidiomycete fungi, ascomycetes, actinomycetes, and some bacteria (Hofrichter, 2002; Baldrian, 2006). The higher availability of substrate (i.e., phenolics) at higher pH is also favorable for MnP and Lac producers (Sinsabaugh, 2010). These suggestions contrast with high LiP activity at low pH (Fig. 3B). LiP is produced only by the specific basidiomycete fungi, Polyporales (e.g., *Phlebia* sp., *Phanerochaete* sp.) (Morgenstern et al., 2008; Kellner et al., 2014). Highly acidic and lignin-rich environments and admixing of woody debris in FH layers are favorable for LiP production (Figs. 3B and 4).

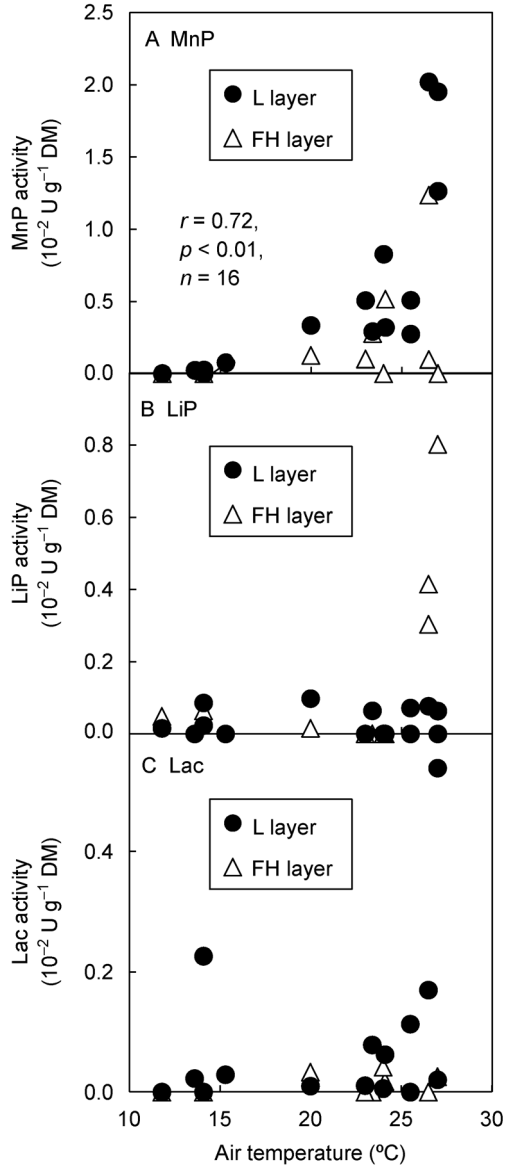

Fig. 2 The activity of manganese peroxidase (A), lignin peroxidase (B), and laccase (C) related to air temperature during the sampling period.

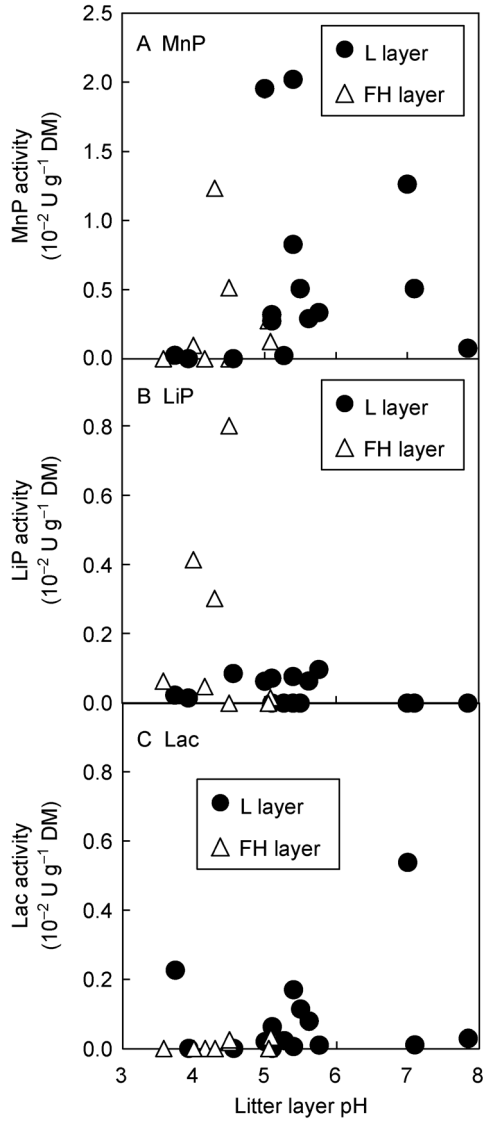

Fig. 3 The activity of manganese peroxidase (A), lignin peroxidase (B), and laccase (C) related to organic layer pH.

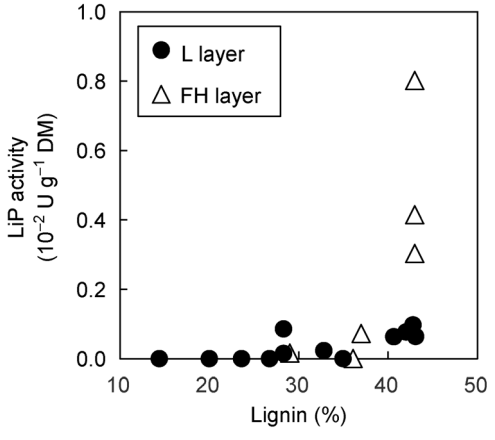

Fig. 4 Lignin peroxidase activity related to litter lignin concentration.

4.3 Pathways of climatic control on ligninolytic enzyme activities and litter carbon turnover

At global climatic scale, litter turnover is more rapid in warmer climates (Fig. 1A), consistent with Trumbore (2000). Litter fragmentation by soil animals (e.g., termite) is known to promote litter C turnover in tropics, but rapid litter turnover ultimately requires efficient lignin degradation by fungal enzymes (Fujii et al., 2018). Significant temperature dependency of lignin degradation is proposed based on the thermodynamic argument that enzymatic reactions decomposing structurally complex, aromatic molecules, such as lignin, require higher activation energy than reactions that

Table 4 Mean (n = 3) ligninolytic enzyme activities in the organic layers in the boreal, temperate, and tropical forests studied.

Site	Location	Vegetation/Soil	Layer	MnP activity (10^{-2} Ug^{-1} DM)	LiP activity (10^{-2} Ug^{-1} DM)	Lac activity (10^{-2} Ug^{-1} DM)
1	Saskatchewan, Canada	Aspen	L	0.08	b.d.l.	0.03
2	Boreal	Aspen	L	0.02	b.d.l.	0.02
3		Jack pine	L	0.02	0.02	0.23
			FH	b.d.l.	0.06	b.d.l.
4		Black spruce	L	b.d.l.	0.09	0.00
5	British Columbia, Canada	Black spruce	L	b.d.l.	0.02	b.d.l.
	Boreal		FH	b.d.l.	0.05	b.d.l.
6	Virginina, USA	*Quercus* spp	L	0.32	b.d.l.	0.06
	Temperate		FH	0.51	b.d.l.	0.02
7	Iwate, Japan	Beech	L	0.33	0.10	0.01
	Temperate		FH	0.51	b.d.l.	0.02
8		Beech	L	0.29	0.06	0.08
			FH	0.28	b.d.l.	b.d.l.
9	Kyoto, Japan	Beech	L	2.02	0.08	0.17
	Temperate		F	1.23	0.30	b.d.l.
			H	0.10	0.41	b.d.l.
10	Ibaraki, Japan	Cedar	L	0.51	b.d.l.	0.11
11	Temperate	Cypress	L	0.27	0.07	b.d.l.
12	Indonesia	Dipterocarp	L	1.95	0.06	0.02
	Tropical		FH	b.d.l.	0.80	0.03
13		*Harpullia arborea* (Oxisols)	L	0.76	b.d.l.	0.50
14		*Harpullia arborea* (Ultisols)	L	1.22	b.d.l.	0.54
15	Cameroon	Mimosaceae (Oxisols)	L	0.51	b.d.l.	0.01
	Tropical		FH	0.10	b.d.l.	b.d.l.
16		Mimosaceae (Ultisols)	L	0.83	b.d.l.	0.01
Literatures						
17	France	*Quercuss ilex*	L	0.60–2.7	b.d.l.	0.0–3.7
18	Japan	*Quercuss serrata*	L	3.56	b.d.l.	b.d.l.
			FH	b.d.l.	0.26	b.d.l.
19	Indonesia	Dipterocarp	L	3.39	b.d.l.	0.09
			FH	b.d.l.	0.88	b.d.l.
20	Thailand	*Lithocarps*	L	0.73	b.d.l.	b.d.l.

b.d.l. = below detection limit. Data sources are Criquet et al. (2000) for Site 17 and Fujii et al. (2013b) for Sites. 18–20.

decompose structurally simpler molecules (Mikan et al., 2002). Such energetics are consistent with the slow turnover of litters with high lignin/N ratios in boreal forests (Fig. 1D; Meentemeyer, 1978). However, fresh litter lignin/N ratios do not fully explain the wide variation in litter C turnover at global scale in our study (Fig. 1D). An increase in MnP activity in warmer climate (Fig. 2A) could be a critical mechanism for efficient lignin degradation.

High Mn/lignin ratios of litter lead to high MnP activities (Fig. 5A) as a control on enzyme activity and litter C turnover, as well as rapid litter turnover (Fig. 1C). A snapshot of enzymatic activities from a single sampling period cannot be extrapolated or related directly with organic layer C or turnover data on annual or longer time scales. Still, high Mn/lignin ratios could promote lignin degradation through stimulating MnP activity. Further, direct effects of warmer climates on elevated microbial and faunal activity correlates with litter decomposition rates that are enhanced by the high Mn availability and MnP activity (Bonnarme and Jeffries, 1990; Berg et al., 2007). The critical roles of MnP in litter turnover at global climatic gradient is supported by the widespread occurrence of MnP in L layers (Table 4; Kellner et al., 2014) and by increased MnP activity and litter Mn/lignin ratios in warmer climates in our study (Fig. 5A). Under the presence of MnP-producing fungi and sufficient H_2O_2 availability, litter Mn/lignin ratios may be a useful indicator of lignin decomposability and could explain rapid litter C turnover in warmer climates from the viewpoint of lignin degradation.

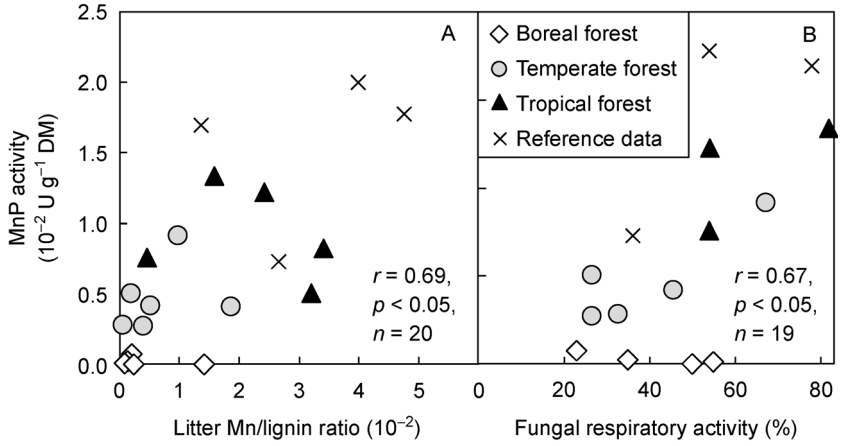

Fig. 5 Manganese peroxidase activity related to fresh litter Mn/lignin ratio (A) and fungal respiratory activity (B). Data are expressed as mass-weighted means for L and FH layers. Reference data are from Criquet et al. (2000) and Fujii et al. (2013b).

5 Conclusions

MnP activity in fresh litter layers increases with increasing air temperature. Fresh litter Mn/lignin ratios correlate positively with MnP activity and contribute to faster litter turnover in warmer climates. The Lac and LiP activities are affected by organic layer and soil pH as well as climate; Lac activity is limited to fresh litter, and LiP activity is limited to acidified and humified layers, respectively. The widespread occurrence of MnP and an increase in MnP activity in warmer climates are reasons for efficient lignin degradation in tropics and during summer seasons. High Mn/lignin ratio in fresh litter could be an indicator of lignin degradability by MnP-producing fungi across global climate gradients. A limited number of data sets were available to support our study, and additional research is warranted for expanding the database and integrating data sets into global patterns of ligninolytic enzyme activity and litter degradation.

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Electronic supplementary material

Supplementary material is available in the online version of this article at https://doi.org/10.1007/s42832-020-0042-6 and is accessible for authorized users.

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