

Influences of nitrogen and phosphorus addition on polyphenol oxidase activity in a forested Andisol

Takashi Kunito · Yoshinori Akagi ·
Ho-Dong Park · Hideshige Toda

Received: 7 September 2008 / Revised: 25 December 2008 / Accepted: 16 March 2009 / Published online: 15 April 2009
© Springer-Verlag 2009

Abstract Increased atmospheric N deposition could suppress plant litter decomposition, due to the P limitation for soil microorganisms in Japanese forested Andisols with a high P sorption capacity. To explore this possibility, we used a laboratory incubation experiment to study the influence of N addition on β -D-glucosidase and polyphenol oxidase activities, which are important for cellulose and lignin degradation, respectively, in an Andisol with larch (*Larix kaempferi*) leaf litter. The addition of N increased the β -D-glucosidase activity, whereas it decreased the polyphenol oxidase activity in the soil. However, the addition of both N and P increased the polyphenol oxidase activity in the soil, suggesting the possibility of; (1) an inferior competitive ability of polyphenol oxidase-producing microorganisms under nutrient-rich conditions and; of (2) their P limitation through competition in the Andisol.

Keywords Lignin · Nitrogen · Phosphorus · Polyphenol oxidase · Andisol

Introduction

The combustion of fossil fuels and use of chemical N fertilizers have released a large amount of reactive N into the atmosphere, leading to large N inputs into the terrestrial environment including forested ecosystems. The increased inputs of anthropogenically derived N may have a significant

impact on the turnover of plant litter and humus in the terrestrial ecosystem (Fog 1988; Berg and Matzner 1997). The effects of N depositions on C cycling in the terrestrial ecosystem, particularly the forest ecosystem, the largest reservoir of terrestrial C (Dixon et al. 1994), have attracted considerable attention, because any change might affect the levels of atmospheric CO₂ that play a central role in controlling climate.

Of all the naturally produced organic chemicals, lignin is probably the most recalcitrant, and is also second only to cellulose in abundance (Kirk and Farrell 1987; Hammel 1997). Thus, the biodegradation of lignin is an important process in the global C cycle. Polyphenol oxidase facilitates the degradation of lignin and other phenolic compounds, and its activity is reported to be the rate-limiting step for the overall decomposition of litter (Carreiro et al. 2000) and humus (Waldrop et al. 2004a). The response of polyphenol oxidase activity in litter and soil to N input is sometimes negative (e.g., Carreiro et al. 2000; Frey et al. 2004; Waldrop et al. 2004b), but is also sometimes positive (e.g., Carreiro et al. 2000; Michel and Matzner 2003; Waldrop et al. 2004b) or neutral (e.g., Carreiro et al. 2000; Michel and Matzner 2003); the detailed mechanism for the variable response to N input is still not clear. In contrast, activation of cellulases (e.g., β -D-glucosidase) that catalyze degradation of the major component of plants, cellulose, often occurs in response to N input (e.g., Waldrop et al. 2004b).

Studies concerning effects of N input on plant litter decomposition have been mostly conducted in the forests of North America and Europe, which have high rates of atmospheric N deposition (e.g., Townsend et al. 1996). Few such studies have been performed in Japan, although some Japanese forest ecosystems also receive considerable inputs of atmospheric N (>10 kg N ha⁻¹ year⁻¹) (Ohruai and Mitchell 1997; Baba and Okazaki 1998). Volcanic ash

Communicated by A. Merino.

T. Kunito (✉) · Y. Akagi · H.-D. Park · H. Toda
Department of Environmental Sciences, Faculty of Science,
Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan
e-mail: kunito@shinshu-u.ac.jp

soils (Andisols), whose high capacity for P sorption might limit biodegradation of plant litter and humus, are widely distributed in Japan (Shoji et al. 1993). According to Ino and Monsi (1964), soil respiration increased by the addition of P in a Japanese Andisol (grassland). Similar results were also reported for Andisols from Colombia and France (Munevar and Wollum 1977; Boudot et al. 1986). In addition, N is sometimes considered as the limiting nutrient for microorganisms in a terrestrial environment (Wardle 1992; Kaye and Hart 1997; Kunito and Nagaoka 2009). Hence, increased atmospheric N deposition could enhance the P limitation in Japanese forested Andisols. In the present study, the influences of N addition on β -D-glucosidase and polyphenol oxidase activities in a forested Japanese Andisol with plant litter were examined in a laboratory incubation study.

Materials and methods

Soil and litter samples

The soil sample (ca. 2 kg) was taken from the A horizon (0–15 cm) of the Andisol, and larch (*Larix kaempferi*) needle-leaf samples from the L horizon in a larch plantation at 1,270 m elevation at the Nishikoma Station, Education and Research Center of Alpine Field Studies, Shinshu University (35.83°N, 137.87°E). The soil sample was sieved through a 2-mm mesh and homogenized well. A portion of the soil was air-dried for chemical analyses, while the remainder was maintained field-moist at 4°C. The soil pH was obtained from a 1:2.5 soil–water suspension. The amounts of organic C and total N were measured with an NC analyzer (YANACO MT-5, Kyoto). A pipet method was used for particle-size analysis. Non-crystalline Fe (Fe_o) and Al (Al_o) were extracted with 0.2 M acid ammonium oxalate at pH 3 in the dark (Blakemore et al. 1981), and the Fe and Al were analyzed by atomic absorption spectrometry (Perkin Elmer 5100ZL, Tokyo). All subsequent data are expressed on a dry weight basis.

The air-dried larch needle leaves were ground with a vibrating sample mill (Heiko Seisakusho TI-100, Tokyo) and then used for the laboratory incubation experiment and litter chemical analyses. The total C and N contents were determined as mentioned above. The total P content in the sample was measured by the vanadomolybdate method (Japan Soil Association 2000) after digestion with nitric acid. The organic materials in the ground litter sample were fractionated into lipids, water-soluble polysaccharides, hemicellulose, cellulose, and lignin at Createrra Inc. (Tokyo), using the proximate analytical method of Waksman and Stevens (1930) with some modifications (Japan Soil Association 2000).

Influence of N addition on β -D-glucosidase and polyphenol oxidase activities

To evaluate the effects of N addition on the β -D-glucosidase and polyphenol oxidase activities in the soil amended with the larch leaf litter, the ground litter (79 mg g⁻¹ soil) and nitrogen (as a solution of ammonium chloride) at two levels (0.57 and 1.14 mg N g⁻¹ soil) were added to the preincubated soil and then mixed well. The soil sample was preincubated at 60% of water holding capacity (WHC) for 1 week at 22°C, and then treated as follows: (1) soil, (2) soil + larch litter, (3) soil + larch litter + lower N, and (4) soil + larch litter + higher N. The treated samples were incubated in loosely capped bottles at 22°C, and distilled water was occasionally added to maintain the soil moisture at a constant level. On days 0, 3, 7, 14, and 28, β -D-glucosidase and polyphenol oxidase activities were determined. The β -D-glucosidase activity was measured using *p*-nitrophenyl- β -D-glucopyranoside as the substrate (Hayano 1992), and polyphenol oxidase using catechol (Perucci et al. 2000). These measurements were done in six replicates.

Influences of N + P and N + C additions on polyphenol oxidase activities

To examine the effects of C and P additions on the polyphenol oxidase activity in the soil, another incubation experiment the same as that described above, except for C and P additions was conducted. The polyphenol oxidase activity was measured 3 days after the following treatments: (1) soil + larch litter, (2) soil + larch litter + N, (3) soil + larch litter + N + C, (4) soil + larch litter + N + P, and (5) soil + larch litter + N + C + P. The amounts of treated C, N and P were intended to approximate the stoichiometry of microbial biomass, and added as sodium acetate (2.9 mg C g⁻¹ soil), ammonium chloride (0.57 mg N g⁻¹ soil), and sodium phosphate (0.12 mg P g⁻¹ soil) according to Allison and Vitousek (2005).

Microbial groups contributing to the soil enzyme activities

The bacterial and fungal contributions to the β -D-glucosidase and polyphenol oxidase activities in the soil were estimated according to Hayano and Tubaki (1985). The soil was oven dried at 105°C for 12 h, and then antibiotics in aqueous solution were added to the soil as follows: (1) no addition, (2) cycloheximide (2 mg g⁻¹ soil), (3) chloramphenicol (1 mg g⁻¹ soil), and (4) cycloheximide (2 mg g⁻¹ soil) + chloramphenicol (1 mg g⁻¹ soil). The content of the added antibiotics was based on the results of our preliminary experiment for the soil (unpublished result).

Distilled water was added to the soil to 60% of the WHC, and a small amount of the untreated moist soil was inoculated for each treatment. After a 7-day incubation at 22°C, the β -D-glucosidase and polyphenol oxidase activities were determined.

Statistical analyses

Repeated measure two-way analysis of variance (ANOVA) was employed to detect significant influences of the treatments and incubation time on the β -D-glucosidase and polyphenol oxidase activities in the soil. Tukey's HSD test, along with one-way ANOVA, were conducted to examine the influence of N addition on β -D-glucosidase and polyphenol oxidase activities at each incubation period, to evaluate the influences of P and C additions on the polyphenol oxidase activities in the soils amended with larch litter and N, and to compare the effects of the antibiotics on the β -D-glucosidase and polyphenol oxidase activities in the soil. A *P* value of less than 0.05 was considered to indicate a statistical significance. All of the statistical analyses were done using the program SYSTAT (version 11; SYSTAT Software).

Results

The characteristics of the soil and larch leaf litter samples are shown in Table 1. The C:N ratio was 51, the lignin:N ratio was 37, and the lignocellulose index [lignin/(holocellulose + lignin)] was 0.64 in the larch leaf litter, indicating that this is high-lignin and recalcitrant litter.

The effects of N addition on the β -D-glucosidase and polyphenol oxidase activities in the soil amended with the larch leaf litter are shown in Fig. 1. The β -D-glucosidase activity increased with the litter addition, and was further increased by N addition in a dose-dependent manner. Activity rapidly increased and reached a plateau at 7 days of incubation for all the three treatments. After 7-day incubation, N addition significantly increased the β -D-glucosidase activity compared to the soil added with litter only ($P < 0.01$), except for the soil added with lower N at 14 days of incubation. In contrast, the polyphenol oxidase activity tended to decrease with litter addition, and further decreased with N addition to the soil, which completely contrasted with the β -D-glucosidase results. The significant influence of N addition on the polyphenol oxidase activity compared to the soil added with litter only ($P < 0.05$) was observed after 3-day incubation, except for the soil added with lower N at 7 days of incubation. The pH was slightly increased (from pH 4.5 to 5.1) in the soil + larch litter sample after the 28-day incubation, while the pH change

Table 1 Properties of soil and larch leaf litter samples (expressed on a dry weight basis)

	Soil	Larch leaf litter
pH	4.5	–
Total C (mg g ⁻¹)	66	540
Total N (mg g ⁻¹)	5.0	11
Total P (mg g ⁻¹)	–	0.67
Texture	Light clay	
Sand (%)	37	
Silt (%)	35	
Clay (%)	28	
Fe _o (mg g ⁻¹)	34	
Al _o (mg g ⁻¹)	31	
Lipids (mg g ⁻¹)		87
Water-soluble polysaccharides (mg g ⁻¹)		76
Hemicellulose (mg g ⁻¹)		116
Cellulose (mg g ⁻¹)		109
Lignin (mg g ⁻¹)		405

Fe_o and Al_o: noncrystalline Fe and Al, respectively, which are extractable with acid ammonium oxalate

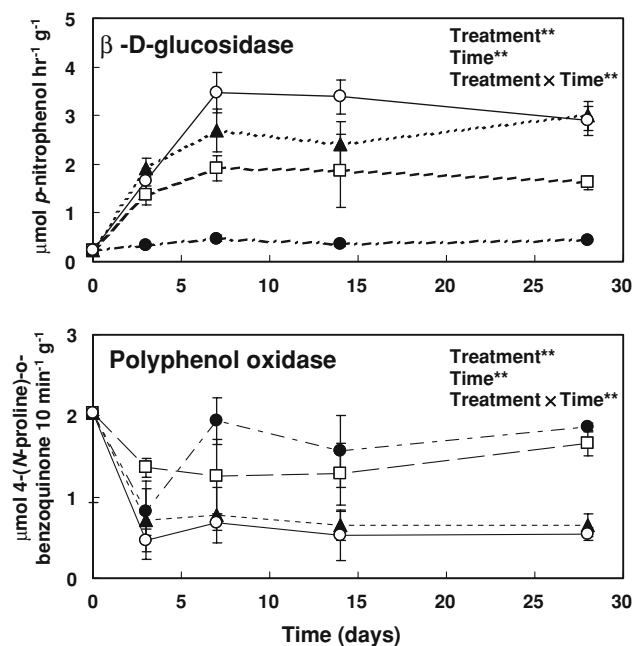


Fig. 1 Variations in β -D-glucosidase and polyphenol oxidase activities during incubation in the soil. Filled circle soil, open square soil + larch leaf litter, filled triangle soil + larch leaf litter + lower N, open circle soil + larch leaf litter + higher N (see details in the text). Each point and error bar represent the mean \pm 1SD. Double asterisk denotes significance at $P < 0.01$ in two-way ANOVA

was less than 0.1 in the other treatments. Therefore, the effects of N addition on the enzyme activities are unlikely to be caused by the pH change in the soil.

To examine the reason why N addition decreased the polyphenol oxidase activity in the soil, the influences of C or P addition as well as N on the enzyme activity were determined (Fig. 2). Addition of N + C had no significant influence on the activity ($P > 0.05$), whereas the addition of N + P significantly increased the polyphenol oxidase activity in the soil ($P < 0.001$). This positive effect of P addition on the activity was also observed in the N + C + P treated soil.

The contributions of bacteria and fungi to the β -D-glucosidase and polyphenol oxidase activities in the soil were evaluated by a selective inhibition method, using the fungicide cycloheximide and the bactericide chloramphenicol (Fig. 3). The production of β -D-glucosidase was drastically inhibited by the cycloheximide treatment, but the activity in the chloramphenicol-treated soil was higher than in the non-treated soil. This result indicates that β -D-glucosidase was exclusively produced by the fungi in the soil. Unlike β -D-glucosidase, the polyphenol oxidase activities were comparable among the non-cycloheximide-, and chloramphenicol-treated soils. The β -D-glucosidase activity showed a 3.6-fold increase in the non-antibiotic treated soil after inoculation of a small amount of moist soil to the heat-treated soil, compared to that in the original soil (Fig. 1). In sharp contrast, the polyphenol oxidase activities in these soils (Fig. 3) were markedly lower (by 86%) than those in the original soil (Fig. 1). These results suggest that the β -D-glucosidase-producing microorganisms might rapidly increase in abundance, whereas the polyphenol

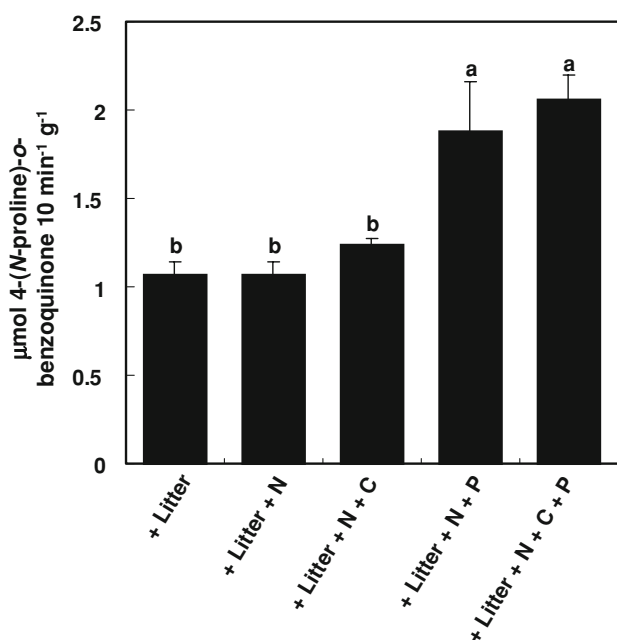


Fig. 2 Influences of N, C and P additions on polyphenol oxidase activity in the soil with larch leaf litter. Each bar and error bar represent the mean \pm 1SD. Values not sharing a common letter are significantly different at $P < 0.001$ in post hoc Tukey's HSD test after significant one-way ANOVA ($P < 0.001$)

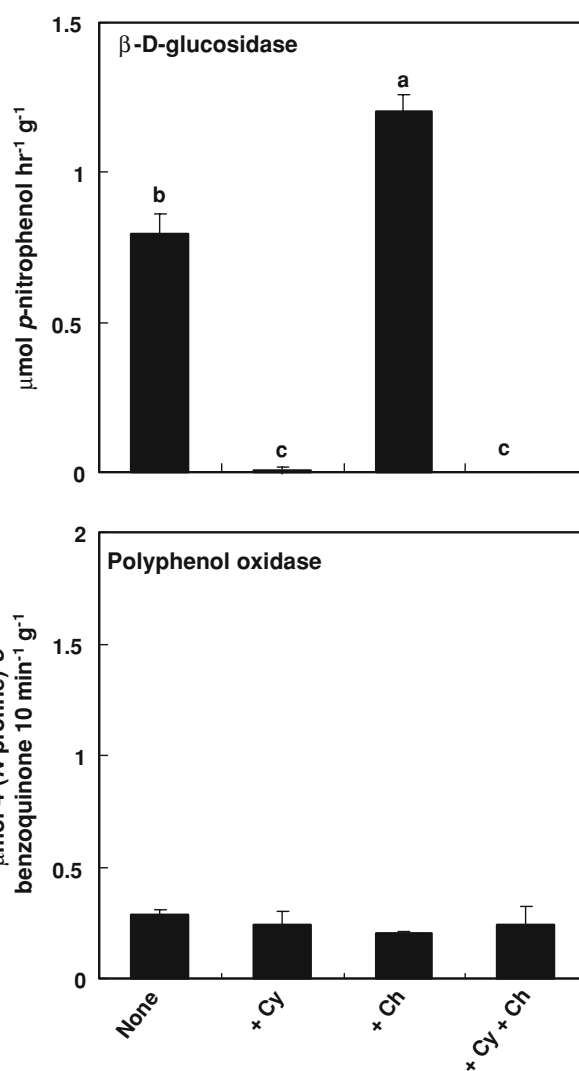


Fig. 3 Inhibition of β -D-glucosidase and polyphenol oxidase activities in the soil treated by fungicide cycloheximide (Cy) and bactericide chloramphenicol (Ch). Each bar and error bar represent the mean \pm 1SD. For the β -D-glucosidase, values not sharing a common letter are significantly different at $P < 0.001$ in post hoc Tukey's HSD test after significant one-way ANOVA ($P < 0.001$). For the polyphenol oxidase, no significant difference was found (one-way ANOVA, $P = 0.34$)

oxidase-producing microorganisms could not compete against the other microorganisms under the high-nutrient condition due to the heat treatment. Alternatively, the production of β -D-glucosidase and polyphenol oxidase was promoted and suppressed, respectively, in the microbial community of the soil.

Discussion

Addition of N caused an increase in the β -D-glucosidase activity, whereas it decreased the polyphenol oxidase

activity in the Andisol amended with larch leaf litter in the present study (Fig. 1). The decrease in the polyphenol oxidase activity with N addition might be due to the recalcitrant nature of the larch leaf litter. According to Waldrop et al. (2004b), polyphenol oxidase activity decreases with N addition in ecosystems with more recalcitrant litter, whereas it increases with N addition in ecosystems with a relatively labile litter. This difference might be due to the fact that ecosystems with more recalcitrant litter contain abundant white rot basidiomycetes, whereas other microorganisms that respond positively to N addition dominate in ecosystems with a relatively labile litter (Waldrop et al. 2004b), and that the production of ligninolytic enzymes is stimulated by N limitation, but suppressed by N sufficiency at least for some white rot basidiomycetes (Kirk and Farrell 1987; Hammel 1997). In contrast, N addition is found to stimulate the β -D-glucosidase activity in an ecosystem-independent manner (Waldrop et al. 2004b). In the present study, however, the decreased polyphenol oxidase activity with N addition throughout the incubation period (28 days) was not likely to be explained solely by the repressed synthesis of polyphenol oxidase in the white rot basidiomycetes, because most of the added N should be immobilized by microorganisms during the incubation. The decrease in the polyphenol oxidase and increase in the β -D-glucosidase activities in the soil might be due to C limitation for microorganisms under excess N, in spite of the litter addition. The microbial community might preferentially expend resources in the form of β -D-glucosidase production rather than polyphenol oxidase production to acquire C from cellulose, which is easier to utilize than lignin. Alternatively, N addition might change the composition of the decomposer community through competition in the soil. If the polyphenol oxidase-producing microbes are competitively inferior for nutrients, especially P, in the Andisol having a high P sorption capacity when larch leaf litter and N are added, then this would cause a decline in their polyphenol oxidase production as well as a decline in their abundance.

To distinguish between these possibilities, the influences of C or P addition as well as N on the polyphenol oxidase activity in the soil were determined (Fig. 2). The addition of C had no significant influence on the activity ($P > 0.05$), suggesting that the suppressed polyphenol oxidase production was not due to C allocation to β -D-glucosidase production rather than to polyphenol oxidase production in the soil microbial community. In contrast, the addition of P significantly increased the polyphenol oxidase activity in the soil ($P < 0.001$). If production of the polyphenol oxidase is physiologically suppressed in white rot basidiomycetes by N addition, the addition of P should not stimulate the polyphenol oxidase activity. Hence, this

result might indicate that the production of polyphenol oxidase is not physiologically suppressed by excess N, and that decomposers of lignin poorly compete under high-nutrient conditions, and therefore, P becomes limiting for these microorganisms in the Andisol. It is reported that fast-growing microbes require a higher amount of P than do slow-growing microbes (Elser et al. 2003). Hence, we expect that the additions of litter and N might select fast-growing microorganisms requiring labile organic matter and also a higher amount of P, and the high P demands of the fast-growing microorganisms might cause the microbial community to be more P limited in the Andisol. Indeed, a rapid decline in the available soil P was observed in the Andisol amended with larch leaf litter (unpublished results). It should be noted that grinding the larch leaf might exacerbate insufficiencies of N and P in the soil by increased utilization of easily degradable C components in the litter (Kunito and Nagaoka 2009).

The polyphenol oxidase activities were not suppressed by either the fungicide or the bactericide (Fig. 3). Polyphenol oxidase is generally considered to be produced by fungi (Kirk and Farrell 1987; Hammel 1997), but laccase, which belongs to a group of polyphenol oxidases, occurs in many bacteria (Alexandre and Zhulin 2000; Claus 2003). Hence, it might be possible that the cycloheximide-resistant fungi or chloramphenicol-resistant bacteria produce this enzyme in the soil. It should be noted that the polyphenol oxidase activities in these heat-treated and inoculated soils (Fig. 3) were markedly lower than those in the original soil (Fig. 1). This result might suggest that the polyphenol oxidase-producing microorganisms poorly competed in the heat-treated soil, which would have contained nutrients derived from heat-killed microorganisms, although it might also be possible that the production of polyphenol oxidase was physiologically suppressed in white rot basidiomycetes by N released from the heat-killed microbes. We prefer the former hypothesis based on the positive effect of P addition on the polyphenol oxidase activity shown in Fig. 2 and general acceptance that ligninolytic microbes are considered to grow very slowly (Moorhead and Sinsabaugh 2006; Osono 2007) and that these microbes predominate at the final stage of litter decomposition (Dix and Webster 1995). Considered together, we assume that the suppressed polyphenol oxidase activity with N addition was probably due to the inferior competitive ability of the polyphenol oxidase-producing microorganisms and their P limitation through competition in the Andisol. Further studies are needed to examine whether the positive effect of P addition on the polyphenol oxidase activity is a general phenomenon in forested Andisols or in ecosystems with recalcitrant litter, or in forested Andisols with recalcitrant litter.

Acknowledgment This study was supported by a Grant-in-Aid for Young Scientists (B) (No. 19710008) from the Ministry of Education, Culture, Sports, Science and Technology, Japan to T.K.

References

- Alexandre G, Zhulin IB (2000) Laccases are widespread in bacteria. *Trends Biotechnol* 18:41–42. doi:10.1016/S0167-7799(99)01406-7
- Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol Biochem* 37:937–944. doi:10.1016/j.soilbio.2004.09.014
- Baba M, Okazaki M (1998) Acidification in nitrogen-saturated forested catchment. *Soil Sci Plant Nutr* 44:513–525
- Berg B, Matzner E (1997) Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environ Rev* 5:1–25. doi:10.1139/er-5-1-1
- Blakemore LC, Searle PL, Daly BK (1981) Soil Bureau Laboratory Methods: methods for chemical analysis of soils. New Zealand Soil Bureau, Scientific Report No. 10A. Department of Scientific and Industrial Research, Lower Hutt, New Zealand
- Boudot JP, Hadj BAB, Chone T (1986) Carbon mineralization in Andosols and aluminium-rich highland soils. *Soil Biol Biochem* 18:457–461. doi:10.1016/0038-0717(86)90053-2
- Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81:2359–2365
- Claus H (2003) Laccases and their occurrence in prokaryotes. *Arch Microbiol* 179:145–150
- Dix NJ, Webster J (1995) Fungal ecology. Chapman and Hall, London
- Dixon RK, Brown S, Houghton RA, Solomon AM, Trexler MC, Wisniewski J (1994) Carbon pools and flux of global forest ecosystems. *Science* 263:185–190. doi:10.1126/science.263.5144.185
- Elser JJ, Acharya K, Kyle M, Cotner J, Makino W, Markow T, Watts T, Hobbie S, Fagan W, Schade J, Hood J, Sterner RW (2003) Growth rate-stoichiometry couplings in diverse biota. *Ecol Lett* 6:936–943. doi:10.1046/j.1461-0248.2003.00518.x
- Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic matter. *Biol Rev Camb Philos Soc* 63:433–462. doi:10.1111/j.1469-185X.1988.tb00725.x
- Frey SD, Knorr M, Parent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *For Ecol Manag* 196:159–171
- Hammel KE (1997) Fungal degradation of lignin. In: Cadisch G, Giller KE (eds) Driven by nature: plant litter quality and decomposition. CAB International, Wallingford, pp 33–45
- Hayano K (1992) Methods of soil enzyme analysis. In: Japanese Society of Soil Microbiology (ed) Methods in soil microbiology. Yokendo, Tokyo, pp 366–376 (in Japanese)
- Hayano K, Tubaki K (1985) Origin and properties of β -glucosidase activity of tomato-field soil. *Soil Biol Biochem* 17:553–557. doi:10.1016/0038-0717(85)90024-0
- Ino Y, Monsi M (1964) On the decomposition rate of soil organic matter in humic allophane soils of Mt. Kirigamine. *Bot Mag* 77:168–175
- Japan Soil Association (2000) Methods of organic matter analysis. Japan Soil Association, Tokyo (in Japanese)
- Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms. *Trends Ecol Evol* 12:139–143. doi:10.1016/S0169-5347(97)01001-X
- Kirk TK, Farrell RL (1987) Enzymatic “combustion”: the microbial degradation of lignin. *Annu Rev Microbiol* 41:465–505. doi:10.1146/annurev.mi.41.100187.002341
- Kunito T, Nagaoka K (2009) Effects of plant litter type and additions of nitrogen and phosphorus on bacterial community-level physiological profiles in a brown forest soil. *Microbes Environ* 24:68–71. doi:10.1264/jisme2.ME08546
- Michel K, Matzner E (2003) Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios. *Biol Fertil Soils* 38:102–109. doi:10.1007/s00374-003-0622-5
- Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. *Ecol Monogr* 76:151–174. doi:10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2
- Munevar F, Wollum AGII (1977) Effects of the addition of phosphorus and inorganic nitrogen on carbon and nitrogen mineralization in Andepts from Colombia. *Soil Sci Soc Am J* 41:540–545
- Ohru K, Mitchell MJ (1997) Nitrogen saturation in Japanese forested watersheds. *Ecol Appl* 7:391–401. doi:10.1890/1051-0761(1997)007[0391:NSIJFW]2.0.CO;2
- Osono T (2007) Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecol Res* 22:955–974. doi:10.1007/s11284-007-0390-z
- Perucci P, Casucci C, Dumontet S (2000) An improved method to evaluate the *o*-diphenol oxidase activity of soil. *Soil Biol Biochem* 32:1927–1933. doi:10.1016/S0038-0717(00)00168-1
- Shoji S, Nanzyo M, Dahlgren RA (1993) Volcanic ash soils. Elsevier, Amsterdam
- Townsend AR, Braswell BH, Holland EA, Penner JE (1996) Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. *Ecol Appl* 6:806–814. doi:10.2307/2269486
- Waksman SA, Stevens KR (1930) A critical study of the methods for determining the nature and abundance of soil organic matter. *Soil Sci* 30:97–116. doi:10.1097/00010694-193008000-00002
- Waldrop MP, Zak DR, Sinsabaugh RL, Gallo M, Lauber C (2004a) Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecol Appl* 14:1172–1177. doi:10.1890/03-5120
- Waldrop MP, Zak DR, Sinsabaugh RL (2004b) Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biol Biochem* 36:1443–1451. doi:10.1016/j.soilbio.2004.04.023
- Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biol Rev Camb Philos Soc* 67:321–358. doi:10.1111/j.1469-185X.1992.tb00728.x