



## OPEN ACCESS

## EDITED BY

Eduardo Vázquez,  
Universidad Politécnica de Madrid, Spain

## REVIEWED BY

Michaela Anna Dippold,  
University of Tübingen, Germany  
Alhassan Idris Gabasawa,  
Ahmadu Bello University, Nigeria

## \*CORRESPONDENCE

Takashi Kunito  
✉ kunito@shinshu-u.ac.jp  
Hirotaka Sumi  
✉ h.sumi@fsc.chubu.ac.jp

RECEIVED 05 December 2025

REVISED 23 January 2026

ACCEPTED 27 January 2026

PUBLISHED 11 February 2026

## CITATION

Sugawa M, Sawada K, Moro H,  
Nagaoka K, Otsuka S, Sumi H and  
Kunito T (2026) Bacterial and fungal  
contributions to the production of  
phosphatases in arable and forest soils.  
*Front. Soil Sci.* 6:1761207.  
doi: 10.3389/fsoil.2026.1761207

## COPYRIGHT

© 2026 Sugawa, Sawada, Moro, Nagaoka,  
Otsuka, Sumi and Kunito. This is an open-  
access article distributed under the terms  
of the [Creative Commons Attribution  
License \(CC BY\)](#). The use, distribution or  
reproduction in other forums is  
permitted, provided the original  
author(s) and the copyright owner(s) are  
credited and that the original publication  
in this journal is cited, in accordance  
with accepted academic practice. No  
use, distribution or reproduction is  
permitted which does not comply with  
these terms.

# Bacterial and fungal contributions to the production of phosphatases in arable and forest soils

Makimi Sugawa<sup>1</sup>, Kozue Sawada<sup>2</sup>, Hitoshi Moro<sup>3</sup>,  
Kazunari Nagaoka<sup>4</sup>, Shigeto Otsuka<sup>5,6</sup>, Hirotaka Sumi<sup>7\*</sup>  
and Takashi Kunito<sup>1\*</sup>

<sup>1</sup>Department of Environmental Science, Faculty of Science, Shinshu University, Matsumoto, Japan,

<sup>2</sup>Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi, Japan, <sup>3</sup>Nagano Agricultural Experiment Station, Suzaka, Japan, <sup>4</sup>Central Region Agricultural Research Center, NARO, Tsukuba, Ibaraki, Japan, <sup>5</sup>Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, <sup>6</sup>Collaborative Research Institute for Innovative Microbiology, The University of Tokyo, Tokyo, Japan, <sup>7</sup>Department of Biological Chemistry, College of Bioscience and Biotechnology, Chubu University, Kasugai, Aichi, Japan

Phosphatases play a key role in the mineralization of organic phosphorus in soils; however, the relative contributions of bacteria and fungi to phosphatase activities remain unclear. Sources of activities of two phosphomonoesterases (acid phosphatase and alkaline phosphatase) and phosphodiesterase in two arable Andisols and two forested Inceptisols were evaluated using selective inhibition with antibiotics. The results showed that all three phosphatases were primarily produced by bacteria in the two arable soils and one forest soil, whereas all three phosphatases were primarily produced by fungi in the forest soil with the lowest pH. These results indicated that fungi can be the primary contributors to alkaline phosphatase production in some soils, challenging the common assumption that bacteria are the main source of alkaline phosphatase activity. Moreover, within a given soil, either bacteria or fungi tend to be the dominant producers of all three phosphatase types, and the identity of the dominant producer appears to be influenced by soil pH. These results highlight the importance of considering the dominant microbial producers when interpreting soil phosphatase activities and organic phosphorus mineralization.

## KEYWORDS

acid phosphatase, alkaline phosphatase, bacteria, fungi, phosphodiesterase

## 1 Introduction

Organic phosphorus represents a substantial proportion of the total phosphorus content in arable and forest soils (1–3) and contributes considerably to plant nutrition after its mineralization to inorganic phosphorus (4). The mineralization of organic phosphorus to inorganic phosphorus is mediated by phosphatases, which are mainly produced by bacteria

and fungi in soils (5), at least outside the rhizosphere. Among these enzymes, acid and alkaline phosphatases differ in their optimal pH ranges and are therefore expected to respond differently to soil pH conditions.

Distinct regulatory mechanisms appear to control the expression of some phosphatase genes in bacteria and fungi (6). Moreover, Rosinger et al. (7) reported that the limiting nutrient can differ for bacteria and fungi in a given soil. Together, these observations suggest that bacteria and fungi may differ not only in their regulation of phosphatase expression but also in their relative contributions to phosphomonoesterase activities under different soil pH conditions. These findings suggest that the level of phosphatase activity in soil may be influenced by whether bacteria or fungi serve as the principal phosphatase producers. Therefore, identifying the source of phosphatase is crucial to obtaining a better understanding of organic phosphorus mineralization in soil.

A comprehensive review by Nannipieri et al. (5) revealed that bacteria are the main source of alkaline phosphatase activity in soil, whereas acid phosphatase can originate from fungi, bacteria, and plants. However, these conclusions were largely based on indirect evidence, such as correlations between microbial community shifts and enzyme activity, or studies of isolated strains. To our knowledge, with the exception of an investigation conducted by Kuroki and Hayano (8), who used selective inhibition to determine that fungi were the primary producers of phosphodiesterase in a soil, no studies have directly identified the sources of soil phosphatases. Thus, the present investigation employed selective inhibition to determine whether bacteria or fungi are the primary source of three phosphatases in arable and forest soils.

## 2 Materials and methods

### 2.1 Soils

We used two arable soils and two forest soils in this study to account for the effects of phosphorus fertilization history and land-use type. Arable soil no. 1 was collected from the Ap horizon at the Nagano Prefecture Vegetable and Ornamental Crop Experiment Station in Shiojiri, Nagano Prefecture, Japan. Arable

soil no. 2 was obtained from the Ap horizon at the NARO Agricultural Research Center for the Hokkaido Region in Sapporo, Hokkaido Prefecture. Both soils were classified as Andisols according to the United States Department of Agriculture (USDA) soil taxonomy. Soil no. 2 was collected from a field that had been left fallow for several years without application of lime or fertilizers. Forest soils 3 and 4 were Inceptisols collected from the A horizon in Minamiaiki and Terasawa, respectively, in Nagano Prefecture. The soil samples were sieved through a 2-mm mesh and stored at 4 °C. A portion of the soil sample was also air-dried for chemical analyses.

### 2.2 Soil analyses

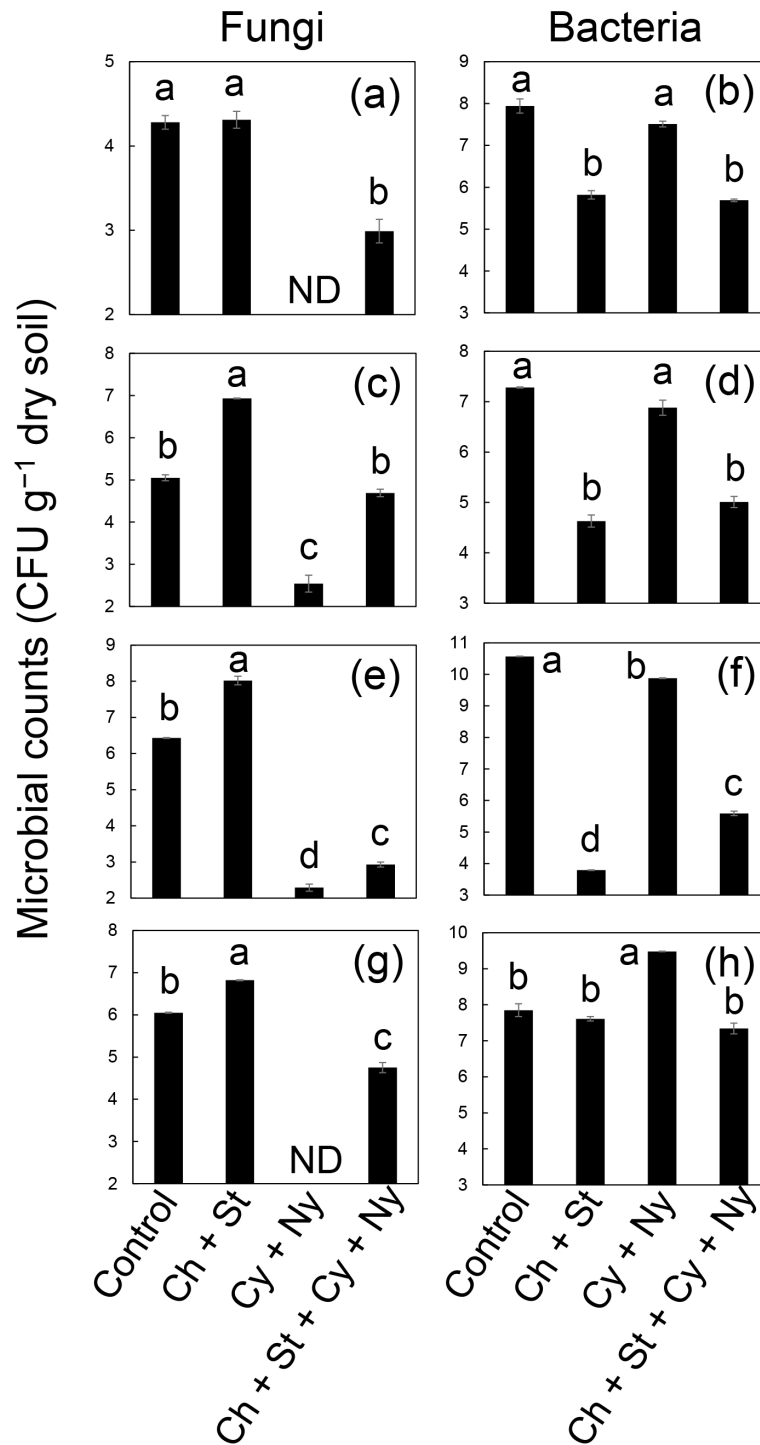
Soil pH was measured in a 1:2.5 soil:solution suspension with distilled water or 1 M KCl. The total C and N were determined using an NC analyzer (JM1000CN, J-Science Lab, Kyoto, Japan). Potentially available P was estimated by the Truog method (9), with Truog-P extracted from a suspension with a 1:200 soil: solution ratio using 0.001 M H<sub>2</sub>SO<sub>4</sub> buffered with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to a pH of 3.0. All concentrations were expressed on a dry weight basis and are shown in Table 1.

### 2.3 Microbial groups contributing to phosphatases production in soils

Bacterial and fungal contributions to phosphatases production in soils were evaluated using the selective inhibition methods developed by Hayano and Tubaki (10), Watanabe and Hayano (11), and Kunito et al. (12), with slight modifications. Briefly, the soil was oven dried at 105 °C for 12 h, after which antibiotics in aqueous solution were added to the soil (15 g on a dry weight basis) as follows (1): no addition (2), cycloheximide (2 mg g<sup>-1</sup> soil) + nystatin (2 mg g<sup>-1</sup> soil) to suppress fungal growth (3), chloramphenicol (1 mg g<sup>-1</sup> soil) + streptomycin (1 mg g<sup>-1</sup> soil) to suppress bacterial growth, or (4) cycloheximide (2 mg g<sup>-1</sup> soil) + nystatin (2 mg g<sup>-1</sup> soil) + chloramphenicol (1 mg g<sup>-1</sup> soil) + streptomycin (1 mg g<sup>-1</sup> soil) to suppress both fungal and bacterial growth. We determined the types and amounts of antibiotics applied in our preliminary experiment. Distilled water was added to the soil to 50% of the water holding capacity, and a small amount of untreated moist soil (0.2 g) was inoculated for each treatment.

TABLE 1 Properties of soil samples studied.

Soil	Soil type	Location	Soil use	pH (H <sub>2</sub> O)	pH (KCl)	Total C	Total N	Truog-P
	(USDA)					(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )
No. 1	Andisol	Shiojiri	Arable	7.2	6.2	43	3.0	213
No. 2	Andisol	Sapporo	Arable	5.5	4.3	50	4.7	51
No. 3	Inceptisol	Minamiaiki	Forest	4.9	4.1	140	10.6	187
No. 4	Inceptisol	Terasawa	Forest	5.8	5.4	132	7.8	25



**FIGURE 1**

Effects of antibiotics addition on microbial counts (mean ± standard error). Microbial counts for fungi in soil No. 1 (a), bacteria in soil No. 1 (b), fungi in soil No. 2 (c), bacteria in soil No. 2 (d), fungi in soil No. 3 (e), bacteria in soil No. 3 (f), fungi in soil No. 4 (g), and bacteria in soil No. 4 (h). Ch, chloramphenicol; St, streptomycin; Cy, cycloheximide; Ny, nystatin; ND, not detected. Bar with the same letter for each soil are not significantly different at  $p = 0.05$ .

After a 7-day incubation at 23 °C, the activities of phosphatases and culturable microbial populations were determined. All samples were incubated in triplicate.

## 2.4 Microbial analyses

The activities of two phosphomonoesterases (acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1)) and phosphodiesterase (EC 3.1.4.1) in soils were measured. Acid and alkaline phosphatase activities were measured using *p*-nitrophenyl phosphate as the substrate in a modified universal buffer (pH 6.5 and 11, respectively) as described by Tabatabai (13). Phosphodiesterase activity was measured using bis-*p*-nitrophenyl phosphate in tris (hydroxymethyl)aminomethane buffer (pH 8.0) (13). It should be noted that the measured activity represents potential rather than *in-situ* activity and reflects the amount of active enzyme present in the soil under substrate-saturating assay conditions (14). Also, because *p*-nitrophenyl phosphate do not cross the cytoplasmic membrane (15), the activities are likely derived extracellular enzymes.

In this study, we considered the possibility that a large amount of DNA derived from microbial residues might remain in the soil after heating treatment at 105 °C. Therefore, we evaluated microbial abundance by plate-count technique instead of quantitative PCR. Culturable bacteria were enumerated by a dilution plate-count technique using a diluted TSB agar plate (tryptic soy broth, 2.0 g; agar, 10 g; cycloheximide, 50 mg per liter) (16). Culturable fungi were enumerated using Martin's rose Bengal streptomycin glucose agar plates (KH<sub>2</sub>PO<sub>4</sub>, 1.0 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g; peptone, 5.0 g; glucose, 10 g; rose Bengal, 33 mg; agar, 20 g; streptomycin, 30 mg per liter) (17). Soil suspensions in sterile tap water (1:10 w/v) were dispersed with a Warring blender (Sakuma Seisakusyo, Japan) at 10,000 rpm for 3 min (18, 19), and the resulting slurry was decimally diluted with sterile tap water.

## 2.5 Statistical analysis

Tukey's HSD test, along with one-way ANOVA, were conducted to compare the effects of the antibiotics on soil phosphatase activities and microbial counts. Goodness of fit to a

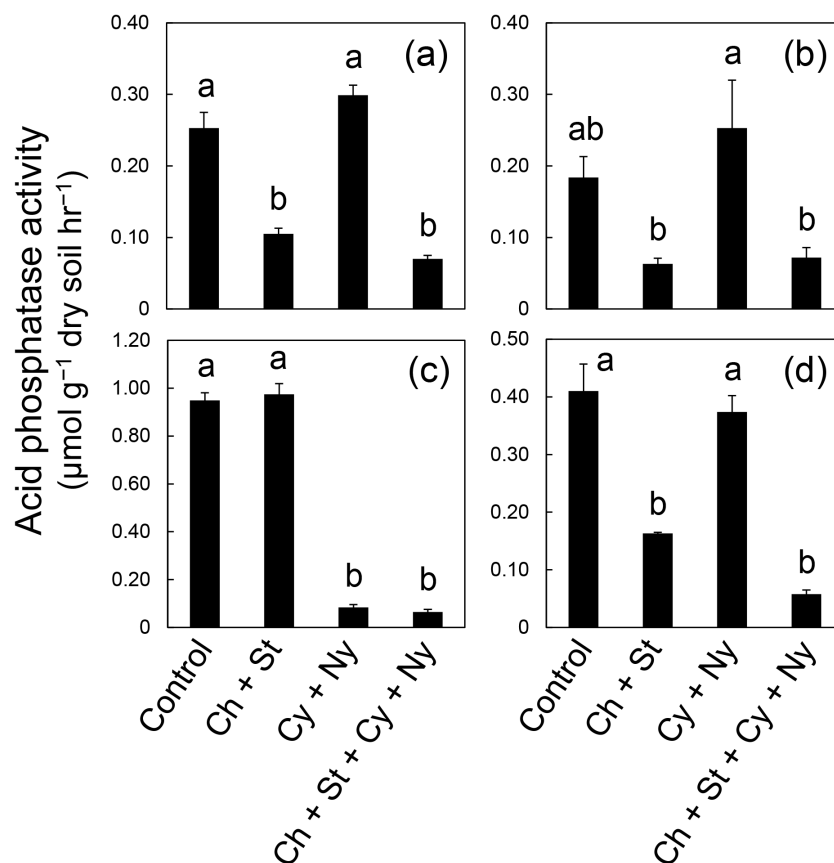


FIGURE 2

Effects of antibiotics addition on acid phosphatase activity (mean ± standard error) in soil No. 1 (a), No. 2 (b), No. 3 (c), and No. 4 (d). Ch, chloramphenicol; St, streptomycin; Cy, cycloheximide; Ny, nystatin. Bar with the same letter for each soil are not significantly different at  $p = 0.05$ .

normal distribution and homogeneity of variances were confirmed using the Shapiro–Wilk test and Bartlett’s test, respectively.

### 3 Results and discussion

#### 3.1 Effects of antibiotic treatments on microbial populations

We used two arable soils and two forest soils in this study. Forest soils had higher organic matter content than arable soils (Table 1). While the addition of cycloheximide + nystatin markedly decreased culturable fungal abundance to near the detection limit ( $p < 0.05$ ), it had only a minor effect on culturable bacterial populations, resulting in bacteria-dominated soil samples (Figure 1). In contrast, the addition of chloramphenicol + streptomycin significantly reduced culturable bacterial abundance ( $p < 0.05$ ) without affecting fungal abundance in all but soil no. 4, leading to fungi-dominated soil samples in all other soils. These findings indicate that selective inhibition using antibiotics successfully established both bacteria- and fungi-dominated soil

samples in this study. The inability to obtain soil samples consisting exclusively of bacteria or fungi is likely due to the ineffectiveness of the added antibiotics against microorganisms located within soil aggregates. It should be noted that the chloramphenicol + streptomycin treatment failed to significantly reduce culturable bacterial abundance in soil no. 4. However, the activities of phosphatases were low in this treatment; thus, the conclusion obtained was not affected. This is discussed in greater detail below. It is also noteworthy that chloramphenicol + streptomycin significantly increased fungi counts, whereas cycloheximide + nystatin significantly increased bacterial counts in some soils. These results are likely explained by the surviving microbial groups proliferating by utilizing the generated microbial necromass as substrates. For example, treatment with chloramphenicol + streptomycin likely caused substantial bacterial death, providing substrates that allowed fungi to grow.

#### 3.2 Bacterial and fungal contributions to phosphatases production in soils

The activities of all three phosphatases (except alkaline phosphatase in soil no. 2) were significantly higher in bacteria-

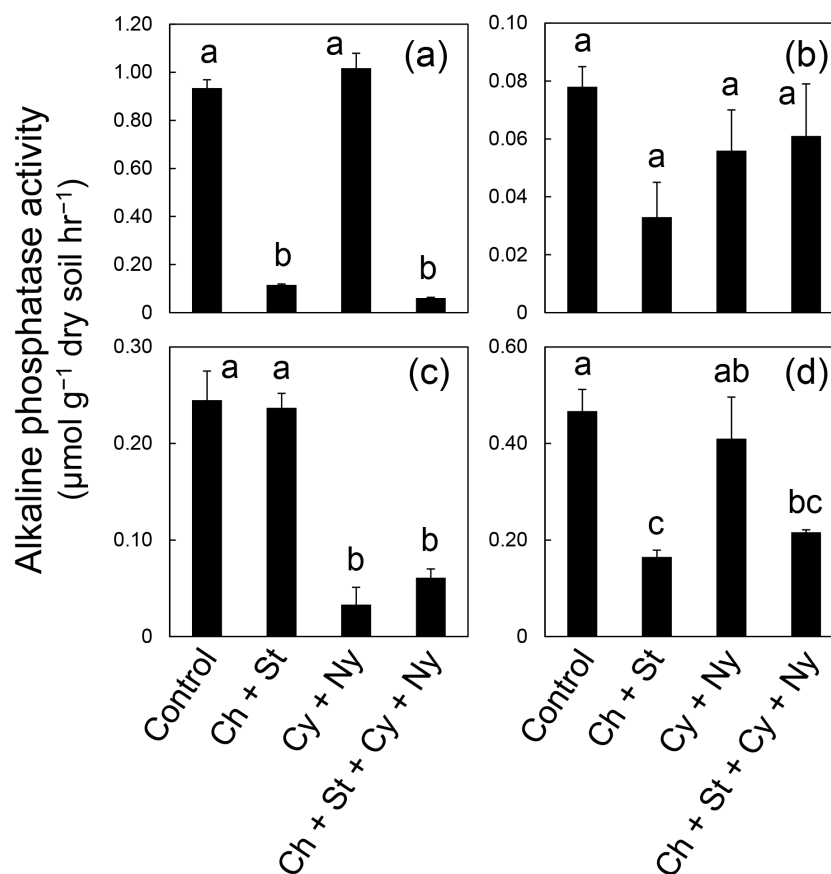


FIGURE 3

Effects of antibiotics addition on alkaline phosphatase activity (mean  $\pm$  standard error) in soil No. 1 (a), No. 2 (b), No. 3 (c), and No. 4 (d). Ch, chloramphenicol; St, streptomycin; Cy, cycloheximide; Ny, nystatin. Bar with the same letter for each soil are not significantly different at  $p = 0.05$ .

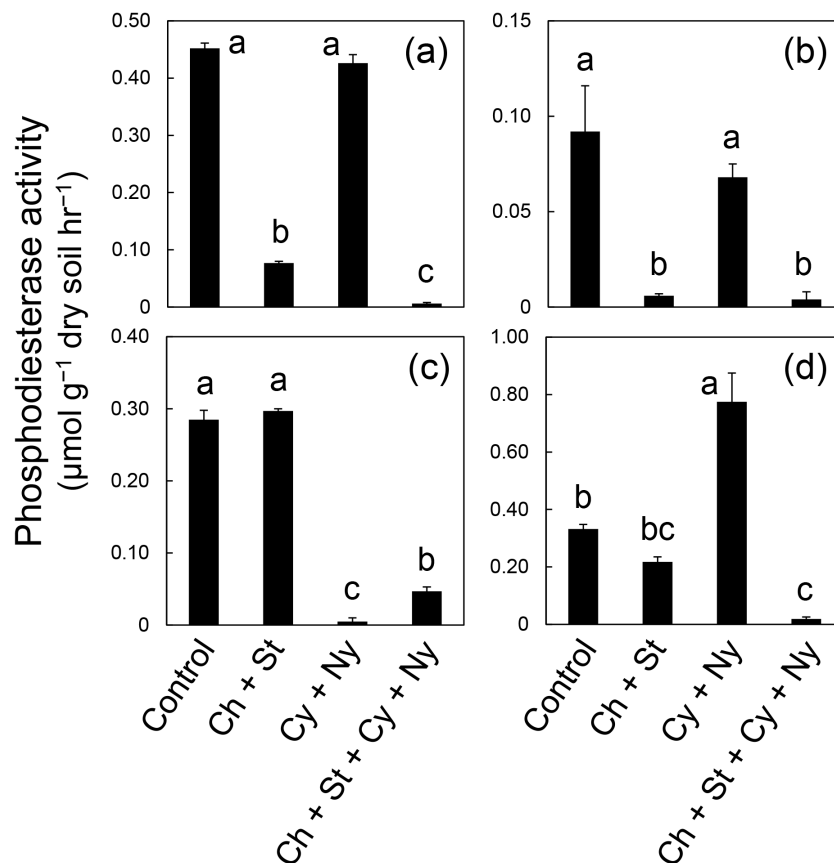


FIGURE 4

Effects of antibiotics addition on phosphodiesterase activity (mean  $\pm$  standard error) in soil No. 1 (a), No. 2 (b), No. 3 (c), and No. 4 (d). Ch, chloramphenicol; St, streptomycin; Cy, cycloheximide; Ny, nystatin. Bar with the same letter for each soil are not significantly different at  $p = 0.05$ .

dominated soil samples treated with cycloheximide + nystatin than in fungi-dominated soil samples treated with chloramphenicol + streptomycin ( $p < 0.05$ ) in soils 1, 2, and 4. Additionally, the activities of these phosphatases were comparable to those of the control without added antibiotics for these three soils (Figures 2–4). These results suggest that bacteria mainly produced all three phosphatases in soils 1, 2, and 4. In contrast, the activities of all three phosphatases were significantly higher in fungi-dominated soil samples treated with chloramphenicol + streptomycin than in bacteria-dominated soil samples treated with cycloheximide + nystatin ( $p < 0.05$ ) in soil no. 3, while they were comparable to those of the control without added antibiotics. This finding suggests that fungi mainly produced all three phosphatases in soil no. 3. A possible explanation for the dominant contribution of fungi to phosphatases production observed only in soil no. 3 might be that this forest soil had the lowest pH among the soils used (Table 1). It is well known that fungi play a more important role than bacteria in organic matter decomposition in acidic forest soils (20). Kuroki and Hayano (8) also reported that the production of phosphodiesterase was primarily attributed to fungi in a forest soil with a pH of 5.5. No effect of Truog-P was observed on whether bacteria or fungi dominated phosphatase production.

While it is generally believed that bacteria are the main source of alkaline phosphatase activity in soils (5), our findings showed that fungi might be the primary contributors in at least some other

soils. Although this study included a limited number of samples, these results indicate that phosphatase production in acidic forest soils (including that of alkaline phosphatase) might be primarily mediated by fungi. The results of this study also indicate that, within a given soil, the same microbial group (either bacteria or fungi) tended to dominate the production of all three phosphatase types. The origins of these phosphatases will need to be confirmed in the future by proteomic analyses. Finally, the identity of the dominant producer might be dependent on soil pH.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## Author contributions

MS: Data curation, Formal analysis, Investigation, Writing – original draft. KS: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. HM: Resources, Supervision, Writing – review & editing. KN: Resources, Supervision, Writing – review & editing. SO: Resources, Supervision, Writing – review &

editing. HS: Conceptualization, Project administration, Supervision, Writing – review & editing. TK: Conceptualization, Project administration, Supervision, Writing – review & editing, Formal analysis, Funding acquisition, Methodology, Resources, Validation, Writing – original draft.

## Funding

The author(s) declared that financial support was received for this work and/or its publication. JSPS KAKENHI Grant Number JP22K05931.

## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

- Kunito T, Tsunekawa M, Yoshida S, Park H-D, Toda H, Nagaoka K, et al. Soil properties affecting phosphorus forms and phosphatase activities in Japanese forest soils: Soil microorganisms may be limited by phosphorus. *Soil Sci.* (2012) 177:39–46. doi: 10.1097/SS.0b013e3182378153
- Moro H, Kunito T, Sato T. Assessment of phosphorus bioavailability in cultivated Andisols from a long-term fertilization field experiment using chemical extractions and soil enzyme activities. *Arch Agron Soil Sci.* (2015) 61:1107–23. doi: 10.1080/03650340.2014.984697
- Fujita K, Kunito T, Moro H, Toda H, Otsuka S, Nagaoka K. Microbial resource allocation for phosphatase synthesis reflects the availability of inorganic phosphorus across various soils. *Biogeochemistry.* (2017) 136:325–39. doi: 10.1007/s10533-017-0398-6
- Moro H, Park H-D, Kunito T. Organic phosphorus substantially contributes to crop plant nutrition in soils with low phosphorus availability. *Agronomy.* (2021) 11:903. doi: 10.3390/agronomy11050903
- Nannipieri P, Giagnoni L, Landi L, Renella G. Role of phosphatase enzymes in soil. In: Bünemann EK, Oberson A, Frossard E, editors. *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling (Soil Biology 26)*. Springer-Verlag, Berlin (2011). p. 215–43.
- Kunito T, Moro H, Mise K, Sawada K, Otsuka S, Nagaoka K, et al. Ecoenzymatic stoichiometry as a temporally integrated indicator of nutrient availability in soils. *Soil Sci Plant Nutr.* (2024) 70:246–69. doi: 10.1080/00380768.2024.2341669
- Rosinger C, Rousk J, Sandén H. Can enzymatic stoichiometry be used to determine growth-limiting nutrients for microorganisms?—A critical assessment in two subtropical soils. *Soil Biol Biochem.* (2019) 128:115–26. doi: 10.1016/j.soilbio.2018.10.011
- Kuroki H, Hayano K. pH curve and origin of phosphodiesterase activity in soil treated with antibiotics causing selective inhibition of microorganisms. *Jpn J Soil Sci Plant Nutr.* (1990) 61:68–73.
- Truog E. The determination of the readily available phosphorus of soils. *J Am Soc Agron.* (1930) 22:874–82. doi: 10.2134/agronj1930.00021962002200100008x
- Hayano K, Tubaki K. Origin and properties of  $\beta$ -glucosidase activity of tomato-field soil. *Soil Biol Biochem.* (1985) 17:553–7. doi: 10.1016/0038-0717(85)90024-0
- Watanabe K, Hayano K. Estimate of the source of soil protease in upland fields. *Biol Fertil Soils.* (1994) 18:341–6.
- Kunito T, Akagi Y, Park H-D, Toda H. Influences of nitrogen and phosphorus addition on polyphenol oxidase activity in a forested Andisol. *Eur J For Res.* (2009) 128:361–6. doi: 10.1007/s10342-009-0271-9
- Tabatabai MA. Soil enzymes. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai MA, Wollum A, editors. *Methods of soil analysis, part 2, microbiological and biochemical properties*. Soil Science Society of America, Madison (1994). p. 775–833.
- Wallenstein MD, Weintraub MN. Emerging tools for measuring and modeling the *in situ* activity of soil extracellular enzymes. *Soil Biol Biochem.* (2008) 40:2098–106. doi: 10.1016/j.soilbio.2008.01.024
- Lidbury IDEA, Borsetto C, Murphy ARJ, Bottrill A, Jones AME, Bending GD, et al. Niche-adaptation in plant-associated Bacteroidetes favours specialisation in organic phosphorus mineralisation. *ISME J.* (2021) 15:1040–55. doi: 10.1038/s41396-020-00829-2
- Kunito T, Saeki K, Oyaizu H, Matsumoto S. Influences of copper forms on the toxicity to microorganisms in soils. *Ecotoxicol Environ Saf.* (1999) 44:174–81. doi: 10.1006/eesa.1999.1820
- Kanazawa S, Kunito T. Preparation of pH 3.0 agar plate, enumeration of acid-tolerant, and Al-resistant microorganisms in acid soils. *Soil Sci Plant Nutr.* (1996) 42:165–73. doi: 10.1080/00380768.1996.10414700
- Kanazawa S, Terashima S, Ohta K. Effect of Waring blender treatment on the counts of soil microorganisms. *Soil Sci Plant Nutr.* (1986) 32:81–9. doi: 10.1080/00380768.1986.10557483
- Kunito T, Saeki K, Nagaoka K, Oyaizu H, Matsumoto S. Characterization of copper-resistant bacterial community in rhizosphere of highly copper-contaminated soil. *Eur J Soil Biol.* (2001) 37:95–102. doi: 10.1016/S1164-5563(01)01070-6
- Binkley D, Fisher RF. *Ecology and Management of Forest Soils. 4th ed.* Chichester: Wiley-Blackwell (2013).

## Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.