agronomy

MDPI

Communication

Organic Phosphorus Substantially Contributes to Crop Plant Nutrition in Soils with Low Phosphorus Availability

Hitoshi Moro [1,2], Ho-Dong Park [1] and Takashi Kunito [1,*]

[1] Department of Environmental Science, Faculty of Science, Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan; morohitoshi@gmail.com (H.M.); pparkhd@shinshu-u.ac.jp (H.-D.P.)
[2] Nagano Prefecture Agricultural Experiment Station, 492 Ogawara, Suzaka 382-0071, Japan
* Correspondence: kunito@shinshu-u.ac.jp

Abstract: To evaluate phosphorus (P) availability and the role of microorganisms in P dynamics in the barley rhizosphere, we constructed a rhizobox using two arable Andosols under different fertilization management regimens and cultivated barley (*Hordeum vulgare* L. cv. Minorimugi) for 5 weeks. The phosphatase-labile pool of organic phosphorus (Po) was assessed using a phosphatase-addition approach in combination with chemical extraction of Po from soils. A considerable amount of inorganic P (Pi) in the $NaHCO_3$ fraction was taken up by barley roots in a soil with high Pi availability, whereas Po, primarily phytate-like P in the $NaHCO_3$ fraction, was hydrolyzed and then taken up by barley roots in a soil with low Pi availability. No significant utilization of either NaOH-Pi or NaOH-Po was observed for both soils during the 5-week cultivation. In the soil with low Pi availability, elevated acid phosphomonoesterase and phosphodiesterase activities, and greater utilization of Po substrates by bacteria in the Biolog ECO plate, were observed in the rhizosphere when compared with those in the bulk soil. This suggested enhanced Po hydrolysis by increased phosphatase activities to meet the P demand, making the Po an important P source for barley in the soil.

Keywords: phosphorus; phosphatase; rhizosphere; Andosol; barley

Citation: 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. https://doi.org/10.3390/agronomy11050903

Academic Editor: Geoff Anderson

Received: 7 April 2021
Accepted: 2 May 2021
Published: 4 May 2021

1. Introduction

Organic phosphorus (Po) comprises a considerable portion of phosphorus (P) in arable Andosols [1,2]. Because Po contributes to crop plant nutrition after its mineralization to inorganic P (Pi) [3], evaluating the lability of soil Po to phosphatase hydrolysis is indispensable for assessing P availability for crop plants in arable soils, in particular Andosols [4]. Because of the remarkably high activity of phosphatase in the rhizosphere, which is produced by both plant roots and microorganisms [5,6], Po seems to be actively mineralized in the rhizosphere. Although the spatial distribution of phosphatase activity in the rhizosphere was revealed using soil zymography [7] and also many studies have suggested the utilization of Po in the rhizosphere by comparing the amount of Po between the rhizosphere and bulk soils [8–10], to our knowledge, no studies have assessed the availability of Po by examining phosphatase-hydrolyzable Po extracted from rhizosphere soils with millimeter order distances from plant roots. In the present study, we assessed Po availability by phosphatase addition to extracts from the rhizosphere of barley cultivated in Andosols using the rhizobox system, which allowed a spatial resolution of 1 mm. Activities of acid phosphomonoesterase and phosphodiesterase and substrate utilization profiles in Biolog plates were determined to investigate the underlying basis of the P dynamics in the rhizosphere.

2. Materials and Methods

2.1. Soil and Rhizobox System

Two soil samples, classified as allophanic Andosols, were collected from the Ap layer in two plots under different fertilization management regimens with mineral fertilizers (N, P, and K were added as $(NH_4)_2SO_4$, $Ca(H_2PO_4)_2 \cdot H_2O$, and KCl, respectively) alone (hereafter "NPK") and with mineral fertilizers and rice straw compost (hereafter "NPK + compost"), at Nagano Prefecture Vegetable and Ornamental Crops Experimental Station in Shiojiri, Nagano Prefecture, Japan. The soil texture (the International Union of Soil Sciences) was light clay with a mean particle size distribution of 22.4% sand, 36.7% silt, and 40.9% clay. Soil samples were air dried and passed through a 0.5-mm sieve after removing plant debris. Soil properties are shown in Table 1.

Table 1. Soil properties.

	NPK Soil	NPK + Compost Soil
pH	6.2	6.3
Total C (g kg^{-1})	34.8	43.4
Total N (g kg^{-1})	2.6	3.1
Total P (mg kg^{-1})	3390	3160

We constructed a rhizobox (Figure S1) to evaluate P dynamics in the barley rhizosphere as described previously [11,12] after applying mineral fertilizer nitrogen (as $(NH_4)_2SO_4$ at 57 mg N kg^{-1} dry soil) and potassium (as KCl at 46 mg K kg^{-1} dry soil). The rhizobox had 15 compartments, each of which were 1-mm thick, 150-mm high, and 150-mm wide; each compartment was separated by a 25-µm nylon mesh. The root compartment was situated in the center of the rhizobox, accommodating seven compartments on each side. Distilled water was added to the soil sample so that it was at 60% water-holding capacity, and this level was maintained during the cultivation by adding distilled water. Seven seeds of barley (*Hordeum vulgare* L. cv. Minorimugi) were sown in the center compartment, and then seedlings were grown for 5 weeks under a 12 h light/dark cycled fluorescent illumination at 17.5 °C in a climate chamber (Biotron LH-200; Nippon Medical and Chemical, Tokyo, Japan). After the cultivation, the rhizobox was dismantled, and the soil was sampled from each compartment. In the root compartment, the root was separated carefully from the soil. The soil samples were stored at 4 °C before determination of soil pH, phosphatase activity, microbial substrate utilization, and phosphorus fractionation. Harvested barley plants were divided into root and shoot by cutting at the soil level, and were dried at 70 °C overnight after washing in distilled water. Ground plant samples were digested using concentrated HNO_3 in Teflon bombs at 100 °C for 1 h. Pi content was measured by the vanadomolybdate method [13].

2.2. Soil Chemical Analyses

Soil pH was measured from a 1:2.5 (*w*/*v*) soil–water suspension. Susceptibility of soil Po to phosphatase hydrolysis was assessed as described previously [14]. In brief, soil samples were sequentially extracted with 0.5 M $NaHCO_3$ (pH 8.5) and with 0.1 M NaOH. After centrifugation and subsequent filtration through a 0.45-µm filter, the pH of both $NaHCO_3$- and NaOH-extracts was adjusted to 5.0. Pi content was determined by the ammonium molybdate-ascorbic acid method [15]. Total P (Pt) content in the extracts was also determined after autoclave persulfate digestion. Three phosphatases were used in the phosphatase hydrolysis experiment [14]: acid phosphatase Type IV-S from potato (PP), acid phosphatase Type I from wheat germ (GP), and nuclease P1 from *Penicillium citrinum* (NP), which were purchased from Sigma (St. Louis, MO, USA). Po was hydrolyzed by adding the enzymes (PP alone, or combination of PP + GP, or combination of PP + GP + NP) to $NaHCO_3$- and NaOH-extracts, followed by incubation at 37 °C for 1 h. After incubation, the Pi content was determined as described above. All analyses were performed in triplicate.

Enzymatically hydrolyzable Po was determined by subtracting the Pi content of the soil extract from that after phosphatase hydrolysis. The concentration of P was expressed as mg P kg^{-1} dry soil. It was shown that PP can hydrolyze simple labile monoester P, the combination of PP + GP can hydrolyze phytate-like P as well as simple labile monoester P, and the combination of PP + GP + NP can hydrolyze DNA-like P in addition to labile monoester P and phytate-like P [14].

2.3. Soil Microbial Analyses

The activities of acid phosphomonoesterase and phosphodiesterase were determined in each compartment of the rhizobox according to [16]. In brief, *p*-nitrophenyl phosphate was used as the substrate in a modified universal buffer of pH 6.5 for acid phosphomonoesterase, and bis(*p*-nitrophenyl) phosphate was used as the substrate in a 0.05 M Tris buffer of pH 8.0 for phosphodiesterase. The activities were expressed on a dry weight basis.

Substrate utilization profiles of soil bacterial and fungal communities were evaluated using Biolog ECO and SFN2 plates (Biolog, Hayward, CA, USA), respectively, as described in [17]. For fungal analysis, the inoculant solution was amended with streptomycin and tetracycline to inhibit bacterial growth. ECO and SFN2 plates were incubated at 28 °C for 72 h and 168 h, respectively. After incubation, absorbances at 595 nm (color development plus turbidity) and 750 nm (turbidity) were measured for the ECO plate, whereas that at 750 nm was measured for the SFN2 plate. For the ECO plate, the effect of turbidity caused by bacterial growth on color development was corrected by subtracting the absorbance at 750 nm from that at 595 nm [18]. Averaged well color development (AWCD) was calculated by dividing the sum of absorbance of all wells by the well number. Relative utilization of Po substrates by bacterial and fungal communities was defined as the ratio of the AWCD of Po substrates (glucose-1-phosphate and D-L-α-glycerol phosphate in the ECO plate, and glucose-1-phosphate, glucose-6-phosphate and D-L-α-glycerol phosphate in the SFN2 plate) to the AWCD of all the substrates contained in the Biolog ECO plate and SFN2 plate, respectively.

All statistical analyses were performed using Python version 3.7.0 (Python Software Foundation, Beaverton, OR, USA). All the data used in figures are included in Table S1.

3. Results and Discussion

3.1. Phosphorus Fractions and Phosphatase-Labile Pool of Po

The dry weight of barley grown in the NPK soil and NPK + compost soil is shown in Table 2. The lower yield in the NPK soil than in the NPK + compost soil is probably because of the low Pi availability, as described below, because sufficient amounts of nitrogen and potassium for barley growth were added to the soils when the rhizobox was prepared.

Table 2. Dry weight and concentration and uptake of phosphorus in shoots and roots of seven barley seedlings.

	Shoot			**Root**		
	Dry weight (g)	P concentration (μg g^{-1})	P uptake (μg)	Dry weight (g)	P concentration (μg g^{-1})	P uptake (μg)
NPK soil	0.60	652	391	1.12	837	937
NPK + compost soil	1.14	566	645	1.69	824	1393

A decline in pH was observed in the root compartment for NPK soil and in the root compartment and its adjacent compartments for NPK + compost soils (Figure 1a). The concentrations of $NaHCO_3$-Pt and -Pi in the NPK soil were significantly lower than those of the NPK + compost soil ($p < 0.01$; Figure 2a,c), which suggested lower P availability in the NPK soil than in the NPK + compost soil. Thus, we regarded the Pi availability of the NPK soil and the NPK + compost soil as being "low" and "high", respectively. In the NPK + compost soil, both $NaHCO_3$-Pt and -Pi concentrations gradually declined toward the

root (r^2 = 0.46, $p < 0.01$; r^2 = 0.75, $p < 0.01$, respectively), but such a relationship was not observed in the NPK soil. These results suggest that uptake of Pi in the $NaHCO_3$ fraction by barley roots was larger in the NPK + compost soil than in the NPK soil. The NaOH-Pt concentration was lower in the NPK soil than in the NPK + compost soil ($p < 0.01$), whereas there was no significant difference in NaOH-Pi concentration between the two soils (Figure 2b,d). Contrary to the results of $NaHCO_3$-Pt and -Pi concentrations, the NaOH-Pt and Pi concentrations did not decline in the rhizosphere in both soils. Thus, NaOH-P seemed not to be a significant source of P for barley in both soils during this short-term cultivation (i.e., 5 weeks).

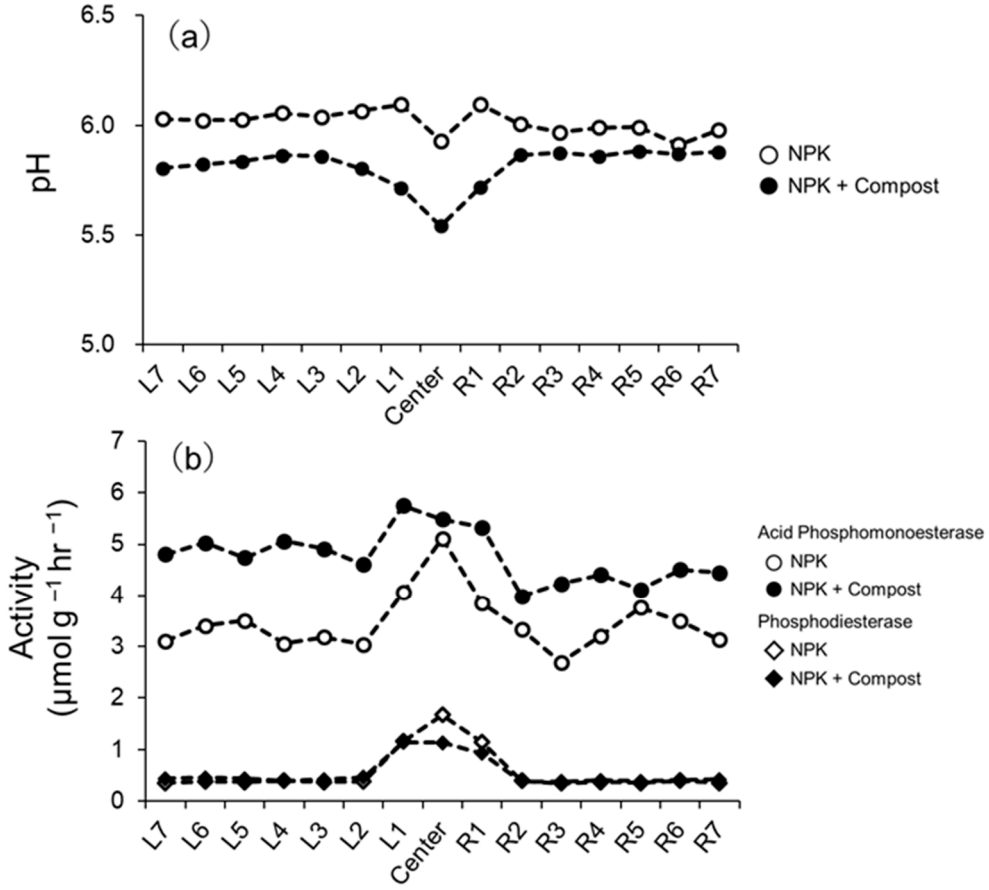

Figure 1. (**a**) Soil pH and (**b**) acid phosphomonoesterase and phosphodiesterase activities in soil compartments collected in 1-mm increments from the root compartment where barley was cultivated for 5 weeks in the rhizobox system.

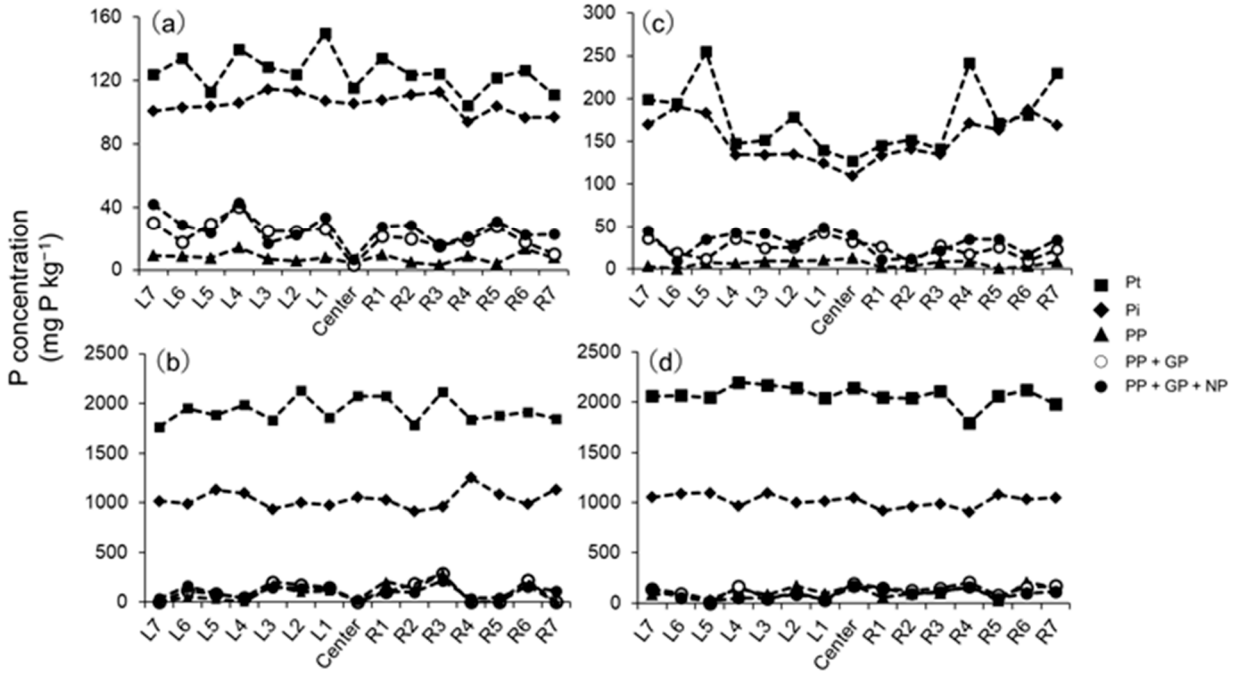

Figure 2. Phosphorus concentrations in (**a**) the $NaHCO_3$ fraction from mineral fertilizer (NPK) soil, (**b**) the NaOH fraction from NPK soil, (**c**) the $NaHCO_3$ fraction from NPK + compost soil, and (**d**) the NaOH fraction from NPK + compost soil in the rhizobox system where barley was cultivated for 5 weeks. PP: acid phosphatase Type IV-S from potato; GP: acid phosphatase Type I from wheat germ; and NP: nuclease P1 from *Penicillium citrinum*.

In the $NaHCO_3$ fraction, PP + GP-hydrolyzable Po and PP + GP + NP-hydrolyzable Po were considerably depleted in the root compartment of the NPK soil (Figure 2a), but no depletion of hydrolyzable Po was observed in the $NaHCO_3$ fraction in the rhizosphere of NPK + compost soil (Figure 2c). This indicated that most of the hydrolyzable Po in the $NaHCO_3$ fraction had been hydrolyzed in the rhizosphere of the NPK soil during the 5-week cultivation. The depletion of hydrolyzable Po in the NPK soil was because of the decrease in GP-hydrolyzable Po. This result implied that phytate—a major form of Po in soil [19]—might be primarily hydrolyzed to Pi in the rhizosphere with the resultant Pi taken up by barley roots in the NPK soil, because the combination of PP + GP could hydrolyze phytate-like P in addition to simple labile monoester P, which could be hydrolyzed by PP alone [14]. The rhizosphere has been suggested to be favorable for phytate hydrolysis [20]. The hydrolysis of phytate-like P in the rhizosphere of the NPK soil was likely performed by phytase from microorganisms, because phytase is synthesized by microorganisms but is not expressed in plant roots [21,22]. In the NaOH fraction, a distinct depletion of phosphatase-hydrolyzable Po was not observed for both soils (Figure 2b,d). We assumed that a decline in PP + GP + NP-hydrolyzable Po in the $NaHCO_3$ fraction of the root compartment compared with those in the adjacent compartments (i.e., L1 and R1 in Figure 2a) was caused by P uptake by barley roots after hydrolysis of Po in the NPK soil; the hydrolyzed Po was estimated to account for 37.3% of total P uptake (1.33 mg P; Table 2) by the barley. Although this might be an overestimate because it did not take into account P incorporation into microbial biomass and also because some portion of hydrolyzed Po might be converted into other P forms, it seems likely that Po, primarily phytate-like P in the $NaHCO_3$ fraction, was a significant source of P for barley cultivated in the NPK soil with low Pi availability. Conceivably, P deficiency might cause increased synthesis and secretion of phosphatases by microorganisms and plant roots, in particular phytase from microorganisms, leading to substantial Po hydrolysis in the rhizosphere of NPK soil. In contrast, Pi in the $NaHCO_3$ fraction was taken up by barley roots in the NPK + compost soil with high Pi availability. Similarly, it was reported that Pi was primarily taken up by barley roots and no significant depletion of Po occurred in the rhizosphere when sufficient P was present in soils, whereas

a significant depletion of Po was observed in the barley rhizosphere in P-deficient soils [23]. It was also reported that available Pi was provided principally through hydrolysis of Po by phosphatases in unfertilized soils [24]. As far as we know, the present study is the first to examine the phosphatase-labile pool of Po in rhizosphere soils with a spatial resolution of 1 mm, providing evidence for the importance of Po for plant nutrition in soils with low Pi availability.

3.2. Phosphatase Activities and Substrate Utilization Profiles of Microbial Communities in the Rhizosphere

The highest activities of both acid phosphomonoesterase and phosphodiesterase were observed in the root compartment and its adjacent compartments in the two soils (Figure 1b). Although acid phosphomonoesterase activity was significantly lower in the NPK soil than in the NPK + compost soil (paired *t* test, $p < 0.01$), the activity in the root compartment was comparable between the two soils. In the case of phosphodiesterase, the activity was not significantly different between the two soils ($p > 0.05$), but the activity in the root compartment was higher in the NPK soil than in the NPK + compost soil. These high activities of the two phosphatases in the root compartment of the NPK soil could be induced by the low Pi availability in this soil (Figure 2); the result was also in agreement with the depletion of Po in the rhizosphere of NPK soil as described above. It is generally accepted that P limitation leads to greater phosphatase activity in soils [25–27].

The AWCD of the bacterial community was significantly higher in the NPK + compost soil than in the NPK soil (Figure 3a; $p < 0.01$), whereas the opposite trend was observed for the fungal community (Figure 3b; $p < 0.01$). In the NPK soil, the AWCD of the bacterial community was extremely elevated in the root compartment, and the AWCD of the fungal community also tended to be higher in the root compartment than in the other compartments. In contrast, such a tendency was not found in the NPK + compost soil. These results suggested increased activity of microorganisms, in particular bacteria, in the rhizosphere of the NPK soil with low Pi availability.

The relative utilization of Po substrates by the bacterial community was remarkably higher in the root compartment than in the other compartments for the NPK soil (Figure 3c), indicating greater utilization of Po by bacteria in the rhizosphere than in the bulk soil of the NPK soil. In contrast, no increase in the relative utilization of Po substrates by the bacterial community was observed in the rhizosphere of the NPK + compost soil. For the fungal community, no enhanced utilization of Po substrates in the rhizosphere was found for both soils (data not shown). These results suggested that bacterial demand for P was greater in the rhizosphere of the NPK soil than in the NPK + compost soil and was also greater than the fungal demand for P in the rhizosphere of both soils. Considering these observations, it appears that microorganisms, mainly bacteria, might produce phosphatases and use phytate-like P in the $NaHCO_3$ fraction to meet the P demand in the rhizosphere of the NPK soil with low Pi availability. A previous report suggested that phytate might be preferentially used by bacteria rather than by fungi in soils [5].

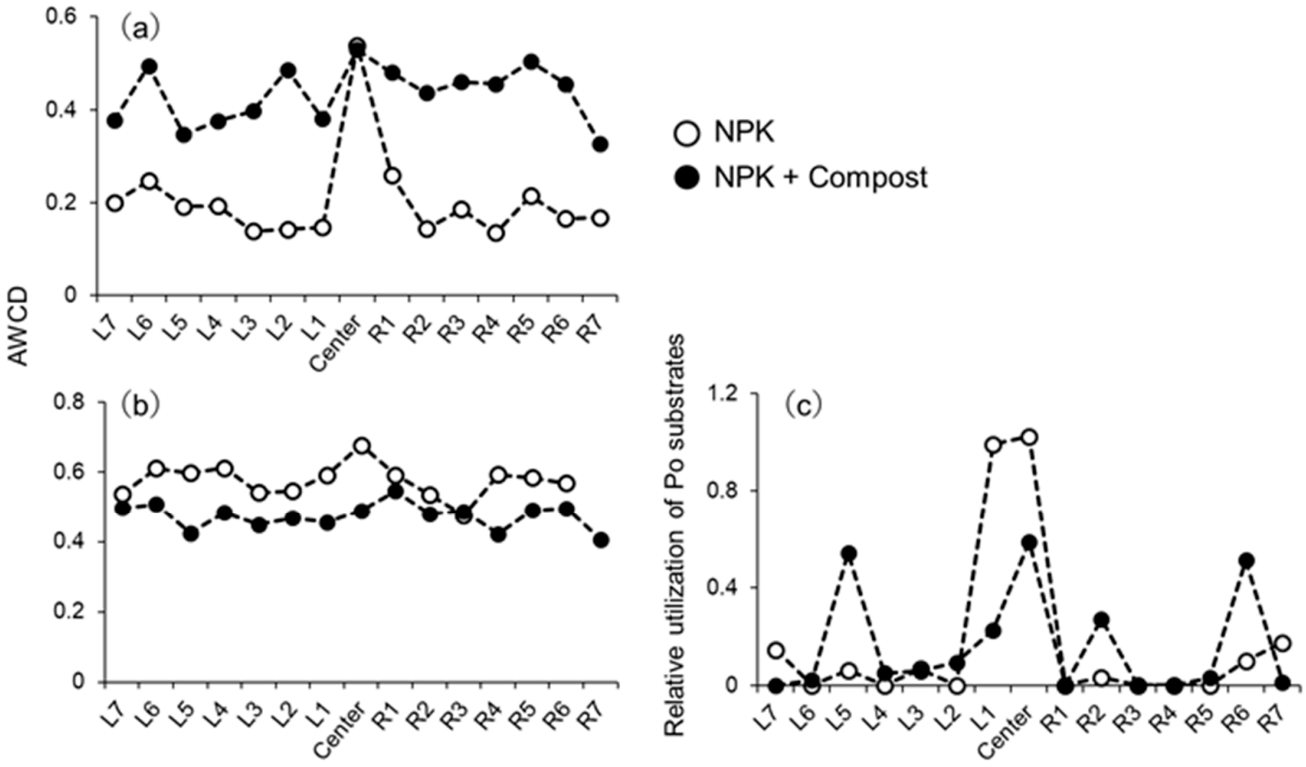

Figure 3. (**a**) Averaged well color development (AWCD) in Biolog ECO plate for the bacterial community, (**b**) AWCD in SFN2 plate for the fungal community, and (**c**) relative utilization of Po substrates by the bacterial community, defined as the ratio of the AWCD of Po substrates to the AWCD of all the substrates contained in the Biolog ECO plate, in soil compartments collected in 1-mm increments from the root compartment where barley was cultivated for 5 weeks in the rhizobox system.

4. Conclusions

This study suggested that a considerable amount of Pi in the $NaHCO_3$ fraction was taken up by barley roots in the NPK + compost soil with high Pi availability, whereas Po, primarily phytate-like P in the $NaHCO_3$ fraction, was hydrolyzed and then taken up by barley roots in the NPK soil with low Pi availability during a 5-week cultivation. Thus, Po seems to be an important P source for crops in soils with low Pi availability. The greater hydrolysis of Po in the rhizosphere of the NPK soil might have been because of greater synthesis of phosphatases by microorganisms, especially bacteria, which was stimulated by low Pi availability. Depletion of the phosphatase-labile pool of Po in the rhizosphere of the NPK soil was found only when we used a phosphatase-addition approach in combination with chemical extraction of Po from soils. This observation suggests the potential usefulness of the phosphatase-addition approach to evaluate Po availability in soils.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11050903/s1: Figure S1: A diagram of rhizobox; Table S1: Data used in Figures.

Author Contributions: Conceptualization: H.M. and T.K.; methodology: H.M.; validation: H.M.; formal analysis: H.M.; investigation: H.M. and T.K.; resources: H.-D.P.; data curation: H.M.; writing—original draft preparation: H.M.; writing—review and editing: H.-D.P. and T.K.; visualization: H.M.; supervision: H.-D.P. and T.K.; project administration: T.K.; funding acquisition: T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number JP24510012.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in supplementary material here.

Acknowledgments: We are grateful to Tsuyoshi Sato (Nagano Prefecture Vegetable and Ornamental Crops Experiment Station, Japan) for providing soil samples.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Borie, F.; Zunino, H. Organic matter-phosphorus association as a sink in P-fixation processes in allophanic soils of Chile. *Soil Biol. Biochem.* **1983**, *15*, 599–603. [CrossRef]
2. 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*, 1107–1123. [CrossRef]
3. Zubillaga, M.S.; Giuffré, L. Soil phosphorus mobilization in different taxonomic orders. *J. Plant Nutr. Soil Sci.* **1999**, *162*, 201–205. [CrossRef]
4. Kunito, T.; Haraguchi, S.; Hanada, K.; Fujita, K.; Moro, H.; Nagaoka, K.; Otsuka, S. pH is the dominant factor controlling the levels of phytate-like and DNA-like phosphorus in 0.5M $NaHCO_3$-extracts of soils: Evaluation with phosphatase-addition approach. *Geoderma* **2021**, *398*, 115113. [CrossRef]
5. Tarafdar, J.C.; Claassen, N. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biol. Fertil. Soils* **1988**, *5*, 308–312. [CrossRef]
6. Nannipieri, P.; Giagnoni, L.; Landi, L.; Renella, G. Role of phosphatase enzymes in soil. In *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling (Soil Biology 26)*; Bünemann, E.K., Oberson, A., Frossard, E., Eds.; Springer: Berlin, Germany, 2011; pp. 215–243.
7. Spohn, M.; Treichel, N.S.; Cormann, M.; Schloter, M.; Fischer, D. Distribution of phosphatase activity and various bacterial phyla in the rhizosphere of *Hordeum vulgare* L. depending on P availability. *Soil Biol. Biochem.* **2015**, *89*, 44–51. [CrossRef]
8. Tarafdar, J.C.; Jungk, A. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biol. Fertil. Soils* **1987**, *3*, 199–204. [CrossRef]
9. George, T.S.; Turner, B.L.; Gregory, P.J.; Cade-Menun, B.J.; Richardson, A.E. Depletion of organic phosphorus from Oxisols in relation to phosphatase activities in the rhizosphere. *Eur. J. Soil Sci.* **2006**, *57*, 47–57. [CrossRef]
10. Waldrip, H.M.; He, Z.; Erich, M.S. Effects of poultry manure amendment on phosphorus uptake by ryegrass, soil phosphorus fractions and phosphatase activity. *Biol. Fertil. Soils* **2011**, *47*, 407–418. [CrossRef]
11. Youssef, R.A.; Chino, M. Development of a new rhizobox system to study the nutrient status in the rhizosphere. *Soil Sci. Plant Nutr.* **1988**, *34*, 461–465. [CrossRef]
12. Sumi, H.; Kunito, T.; Ishikawa, Y.; Sato, T.; Park, H.D.; Nagaoka, K.; Aikawa, Y. Plant roots influence microbial activities as well as cadmium and zinc fractions in metal-contaminated soil. *Chem. Ecol.* **2015**, *31*, 105–110. [CrossRef]
13. Kitson, R.E.; Mellon, M.G. Colorimetric determination of phosphorus as molybdivanadophosphoric acid. *Ind. Eng. Chem. Anal. Ed.* **1944**, *16*, 379–383.
14. He, Z.; Griffin, T.S.; Honeycutt, C.W. Enzymatic hydrolysis of organic phosphorus in swine manure and soil. *J. Environ. Qual.* **2004**, *33*, 367–372. [CrossRef] [PubMed]
15. Murphy, J.; Riley, J.P. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **1962**, *27*, 31–36.
16. Tabatabai, M.A. Soil enzymes. In *Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties*; Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A., Eds.; Soil Science Society of America: Madison, WI, USA, 1994; pp. 775–833.
17. Classen, A.T.; Boyle, S.I.; Haskins, K.E.; Overby, S.T.; Hart, S.C. Community-level physiological profiles of bacteria and fungi: Plate type and incubation temperature influences on contrasting soils. *FEMS Microbiol. Ecol.* **2003**, *44*, 319–328. [CrossRef]
18. Kunito, T.; Nagaoka, K. Effects of plant litter type and additions of nitrogen and phosphorus on bacterial community-level physiological profiles in a brown forest soil. *Microbes Environ.* **2009**, *24*, 68–71. [CrossRef] [PubMed]
19. Turner, B.L.; Papházy, M.J.; Haygarth, P.M.; McKelvie, I.D. Inositol phosphates in the environment. *Phil. Trans. R. Soc. Lond. B* **2002**, *357*, 449–469.
20. Menezes-Blackburn, D.; Jorquera, M.A.; Greiner, R.; Gianfreda, L.; Mora, M.L. Phytases and phytase-labile organic phosphorus in manures and soils. *Crit. Rev. Environ. Sci. Technol.* **2013**, *43*, 916–954. [CrossRef]
21. Richardson, A.E.; Hadobas, P.A.; Hayes, J.E.; O'Hara, C.P.; Simpson, R.J. Utilization of phosphorus by pasture plants supplied with *myo*-inositol hexaphosphate is enhanced by the presence of soil micro-organisms. *Plant Soil* **2001**, *229*, 47–56. [CrossRef]
22. Greiner, R. Phytate-degrading enzymes: Regulation of synthesis in microorganisms and plants. In *Inositol Phosphates: Linking Agriculture and the Environment*; Turner, B.L., Richardson, A.E., Mullaney, E.J., Eds.; CAB International: Wallingford, UK, 2007; pp. 78–96.

23. Ye, D.; Zhang, X.; Li, T.; Xu, J.; Chen, G. Phosphorus-acquisition characteristics and rhizosphere properties of wild barley in relation to genotypic differences as dependent on soil phosphorus availability. *Plant Soil* **2018**, *423*, 503–516.
24. Sharpley, A.N. Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil Sci. Soc. Am. J.* **1985**, *49*, 905–911. [CrossRef]
25. Kunito, T.; Tobitani, T.; Moro, H.; Toda, H. Phosphorus limitation in microorganisms leads to high phosphomonoesterase activity in acid forest soils. *Pedobiologia* **2012**, *55*, 263–270. [CrossRef]
26. 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*, 325–339. [CrossRef]
27. Mise, K.; Maruyama, R.; Miyabara, Y.; Kunito, T.; Senoo, K.; Otsuka, S. Time-series analysis of phosphorus-depleted microbial communities in carbon/nitrogen-amended soils. *Appl. Soil Ecol.* **2020**, *145*, 103346. [CrossRef]