applied microbiology

MDPI

Article

Moderate Phosphorus Addition to Field-Grown Bananas Enhanced Soil Microbial Enzyme Activities but Had Negligible Impacts on Bacterial, Fungal, and Nematode Diversity

Anna-Belle C. Clarke [1], Hazel R. Lapis-Gaza [2], Stuart Irvine-Brown [3], Rebecca Lyons [1], Jiarui Sun [1], Anthony B. Pattison [1,2] and Paul G. Dennis [1,*]

[1] School of Environment, The University of Queensland, Turrbal Country, Brisbane, QLD 4072, Australia; tony.pattison@daff.qld.gov.au (A.B.P.)
[2] Department of Agriculture and Fisheries, Centre for Wet Tropics Agriculture, 24 Experimental Station Road, South Johnstone, QLD 4859, Australia; hazel.gaza@daf.qld.gov.au
[3] Department of Agriculture and Fisheries, Maroochy Research Facility, 47 Mayers Road, Nambour, QLD 4560, Australia; stuart.irvinebrown@daf.qld.gov.au
* Correspondence: p.dennis@uq.edu.au

Abstract: On commercial banana (*Musa* spp.) plantations, soils are often supplemented with phosphorus (P) fertiliser to optimise production. Such additions may influence the diversity and function of soil microbial communities, which play important roles in P cycling and affect plant fitness. Here, we characterised the effects of P addition on the diversity and function of banana-associated microbial communities. P addition was associated with significant increases in soil P and the activities of alpha-glucosidase, chitinase, arylsulphatase, and acid phosphatase, but not beta-glucosidase or xylosidase. P addition also expedited bunch emergence and harvest, but did not influence fruit yield, plant height, or foliar P. There were no significant effects of P addition on the alpha or beta diversity of bacterial, fungal, and nematode communities, including members of the core microbiome. The only exceptions to this was an increase in the relative abundance of a *Fusarium* population in roots. These results indicate that phosphorus application to banana soils may stimulate microbial enzyme activities with minor or negligible effects on microbial diversity.

Keywords: microbiome engineering; *Fusarium wilt*; bacteria; fungi; nematodes; rhizosphere

Citation: Clarke, A.-B.C.; Lapis-Gaza, H.R.; Irvine-Brown, S.; Lyons, R.; Sun, J.; Pattison, A.B.; Dennis, P.G. Moderate Phosphorus Addition to Field-Grown Bananas Enhanced Soil Microbial Enzyme Activities but Had Negligible Impacts on Bacterial, Fungal, and Nematode Diversity. *Appl. Microbiol.* **2024**, *4*, 1582–1599. https://doi.org/10.3390/applmicrobiol4040108

Academic Editor: Ian Connerton

Received: 14 November 2024
Revised: 27 November 2024
Accepted: 28 November 2024
Published: 29 November 2024

1. Introduction

Bananas (*Musa* spp.) are a popular commodity with global production exceeding 100 million tonnes annually [1]. They serve as a staple food in many low-income countries and significantly contribute to the gross domestic product of South and Central American, Asian, and African nations [2]. To achieve competitive yields, commercial plantations typically use large quantities of agrochemicals, including fertilisers and biocides. Poor phosphorus (P) nutrition in banana stunts plant growth and root development, with older leaves displaying marginal chlorosis and a characteristic 'saw-tooth' necrotic effect, and younger leaves adopting a blueish-green appearance [3]. While bananas have a low demand for P in comparison to other horticultural crops, its addition to young, developing plants can improve production [3]. Queensland banana farms are advised to limit application to $\leq$60 kg P ha^{-1} yr^{-1} to minimise nutrient pollution within Great Barrier Reef catchments [4,5]. Furthermore, P application may also modify the structure of plant and soil microbiomes, which influence host fitness via a range of mechanisms including modulation of nutrient availability, the release of phytohormones, and defence against plant pests and pathogens [6]. Hence, understanding how P addition influences *Musa* spp. microbiomes will help to identify best management practices for sustainable banana production. For example, if P addition significantly alters microbial community composition, it may be considered a useful lever to manage microbiome to enhance production.

In crops other than banana, studies have investigated the effects of P addition relative to mock treated soils. In one case, the diversity and composition of bacterial and fungal communities in bulk and rhizosphere soil was observed to be similar between treatments with only minor differences detected for individual lineages [7]. At high levels of P (>100 kg ha^{-1}) addition, however, Pantigoso et al. [8] observed significant changes in the composition of rhizosphere bacterial communities. P addition can also influence the richness of arbuscular mycorrhiza fungi [9], which are known to improve banana growth and P uptake [10]. For nematodes, P addition was found to have no significant effect on diversity [11]. In addition, P amendment has been shown to reduce potential phosphatase, chitinase, and xylosidase [12,13], but not alpha and beta-glucosidase activities [14]. In banana plantation soils, available P has been observed to be negatively correlated with the relative abundances of *Proteobacteria* (now *Pseudomonadota*), *Anaerotruncus*, and *Nitrolancea* populations [15] and positively correlated with members of the *Chloroflexi* (now *Chloroflexota*) and *Acidobacteria* (now *Acidobacteriota*) [16]. While these purely correlative findings suggest that P availability may influence bulk soil microbiomes in banana plantations, we are not aware of any studies that have tested the validity of these associations through experimental manipulation of P concentration. Furthermore, we are not aware of other studies that have considered the effects of P addition on the diversity and function of microbial communities associated with banana plant compartments, such as the rhizosphere.

Previously, we have identified 36 core bacteria [17] and 21 core fungi [18], which, due to their persistent associations with *Musa* spp., are thought to play a disproportionately large role in influencing host fitness. Having identified core taxa, a logical next step is to improve understanding of their ecological preferences, such that management approaches can be designed to manipulate their abundances and activities. As P addition is a common management practice on farms, it is useful to consider whether it could be used as a lever to manage banana microbiomes and the frequencies of core taxa.

Here, we characterised the effects of P addition on banana soil- and root-associated microbial communities in a field experiment over two years. We determined the potential activities of arylsulphatase, chitinase, alpha- and beta-glucosidase, phosphatase, and xylosidase. The diversity and composition of bacterial and fungal communities was assessed using 16S rRNA gene and ITS2 gene amplicon sequencing, respectively. In addition, we identified and investigated the impacts of P addition on OTUs matching the 36 core bacteria and 21 core fungi of banana associated with our previous work [17,18]. Lastly, the diversity and frequencies of nematodes were assessed using microscopy. We tested the hypotheses that P addition would increase the diversity and potential activity of banana plant- and soil-associated microbiomes.

2. Materials and Methods

2.1. Design of Field Experiment

The field experiment was at the South Johnstone Research Station, in Far North Queensland, Australia (17°36′19″ S, 145°59′55″ E). The soil at the site is a dermosol [19], with clay loam texture (35 ± 2.6% clay, 27 ± 0.7% silt, and 38 ± 3% sand). The experiment was established in January 2019, from tissue culture banana plantlets (*Musa* AAA (Cavendish sub-group) 'Williams'), as a randomised block design of 10 plots (two treatments × five replications). Each plot consisted of eight banana plants in a single row with 2.55 m spacing between plants. As banana plants have a high demand for N, both control (Ctrl) and treatment (P) plots received 400 kg N ha^{-1} yr^{-1} as urea via fertigation, except after planting, when the relatively small plants received 100 kg N ha^{-1} yr^{-1} [20]. Treatment (P) plots were fertilised with 50 kg P ha^{-1} yr^{-1} single super phosphate (SSP) in the first year, and 20 kg P ha^{-1} yr^{-1} mono-ammonium phosphate (MAP) and 20 kg P ha^{-1} yr^{-1} SSP in the second year (Figure 1). Slightly more phosphorus is added in the first year, as banana plants initially require higher amounts, but less is needed in subsequent ratoon crops (those that develop from a sucker after the previous plant has been harvested) due to phosphorus redistribution within the plant [3]. Control (Ctrl) plots received no P application over the

two years. Crop management followed standard commercial practices, with irrigation used as required, herbicides applied for weed control, excess suckers removed leaving a single 'following' sucker for the next ratoon, and leaf diseases managed through a combination of leaf removal and fortnightly applications of fungicides or as required [21].

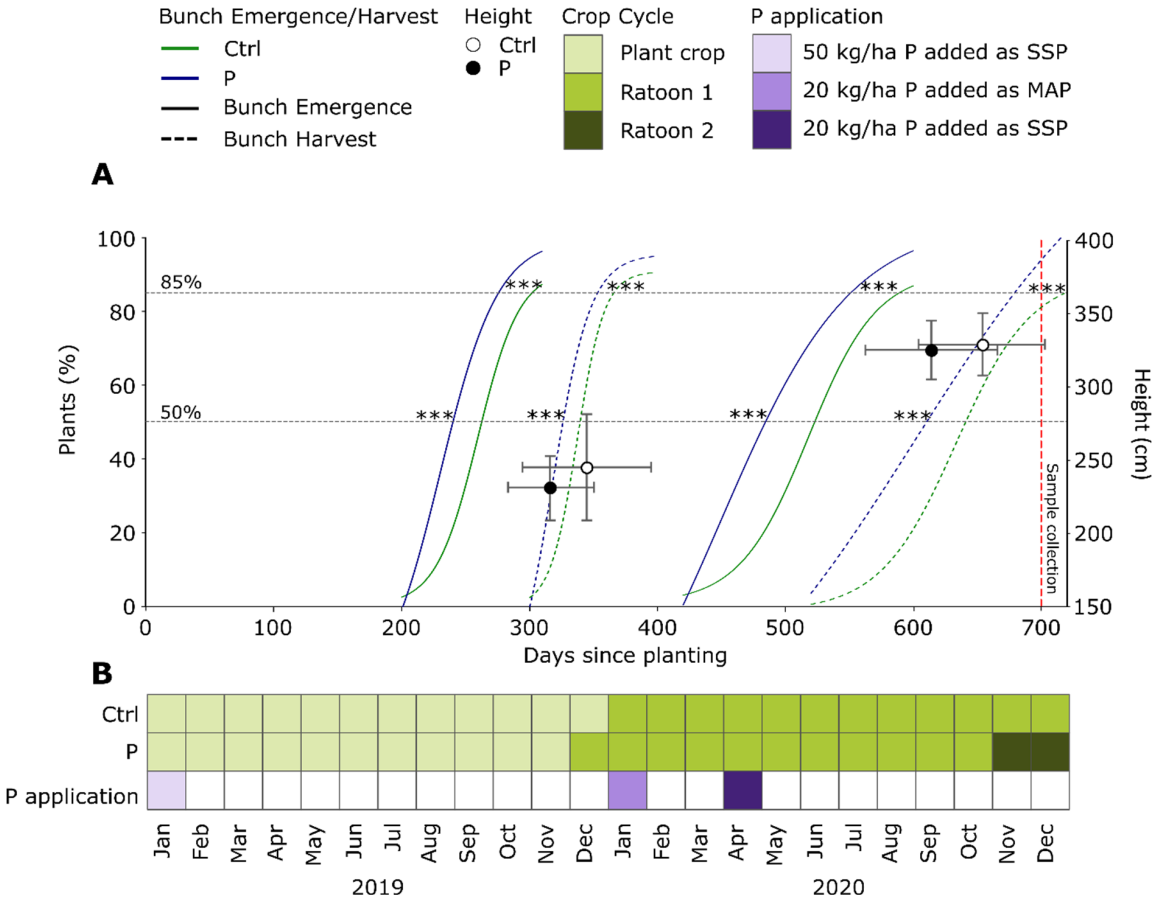

Figure 1. Timeline highlighting key attributes of (**A**) plant performance and (**B**) treatment applications. In Panel A, curves represent the percentage of plants (y axis 1) with emerged (solid lines) or harvested (dotted lines) bunches in control (green) or elevated P (blue) plots, as predicted using logistic regression. Asterisks highlight significant differences between treatments (Ctrl vs. P) when 50% and 85% of plants have reached bunch emergence or harvest ($p \leq 0.001$ ***). The mean height of plants (y axis 2) when bunches were harvested is represented by white (control) and black (elevated P) circles, with error bars representing the standard error of the means. Lastly, the verticle red dotted line highlights when samples were collected for microbiological characterisation. In Panel B, the timeline depicts crop cycle (shades of green) and P application (shades of purple). A ratoon is the subsequent crop from a sucker plant after the previous plant has been harvested. Here, we show a new ratoon starting when ≥85% of the plants have been harvested.

2.2. Plant Performance

Plant performance was assessed by measuring (1) the time from planting until banana bunch emergence and bunch harvest, (2) plant height at bunch harvest, and (3) the weight of banana bunches at harvest. Due to the inherent variability of banana growth, a crop cycle or 'ratoon' for a treatment was deemed to have been completed when 85% of bunches had been harvested. A logistic regression model was fitted to the cumulative bunch emergence and harvest data within each treatment, to compare crop cycle duration between control and elevated P plots. The logistical model followed

$$y(t) = C + \frac{A}{1 + e^{(-B(x-M))}}$$

where $y(t)$ is the value of the function at time t; A is the upper asymptote, representing the maximum value that $y(t)$ approaches over time; B is a growth rate constant; M is a time shift parameter, indicating the point in time when the growth is fastest; and C is a constant, representing the lower asymptote or baseline value.

2.3. Sample Collection

Root and bulk soil samples were collected in December 2020, after the experiment had run for two years. Bulk soil samples were collected 5 cm from the base of all plants (n = 8) in each plot using a sterile trowel to a depth of 10 cm. All bulk soils were taken in line with the following sucker and the mother plant and then pooled into a composite sample per plot for soil chemistry, enzyme activity, nematode counts, and DNA metabarcoding analyses. Three plants were then sampled per plot to make a composite root sample for DNA metabarcoding. Roots were selected from three different points around each plant and removed from the soil using sterilised scissors. They were then shaken to remove bulk soil and sections from the base, middle, and apex were sampled from each root. Points that had been handled, albeit with gloves, were then cut away so only unhandled root sections remained. Leaf samples were collected throughout the experiment for foliar P determination as previously described [22]. Samples were stored on ice in the field and then transferred to a −20 °C freezer for DNA extraction and chemical analyses, or a 4 °C cold room for enzyme analyses, which were performed within 1–5 days of sampling. Prior to DNA extraction and chemical analyses, samples were lyophilised using a freeze drier.

2.4. Soil Chemical and Biochemical Measurements

Soil physicochemical properties were analysed from freeze-dried bulk soil samples by Nutrient Advantage Laboratory (Werribee, Victoria), using methods described in Rayment and Lyons [23]. The properties measured included pH, electrical conductivity (1:5 soil:water), organic C (Walkley–Black), phosphorus buffering index, total C, nitrate nitrogen, and Colwell P. Potassium permanganate oxidisable C (labile C) was determined following the method described by Moody and Cong [24]. Organic matter was calculated as an estimate from organic carbon, based on the calculations previously described [25]. The potential activities of alpha-glucosidase, beta-glucosidase, and chitinase were measured to investigate bacterial involvement in the carbon cycle. Similarly, xylosidase activity was assessed for its role in the nitrogen cycle, acid phosphatase for the phosphorus cycle, and arylsulphatase for the sulphur cycle. These analyses were performed using the microplate-scale fluorometric method described in Lapis-Gaza and Pattison [26]. Foliar P data were assessed using inductively coupled plasma (ICP) spectrometry on freeze-dried leaf samples as previously described [27].

2.5. Characterisation of Nematode Communities

Nematodes were extracted from bulk soil samples using a modified Baermann funnel technique [28]. Briefly, mesh baskets containing a single sheet of tissue paper and 200 g soil were placed in metal trays with 250 mL of deionised water. After 48 h, the soil was discarded, and the solution was passed through a 25 µm sieve and backwashed twice with 10 mL of deionised water to collect the nematodes. From each 20 mL soil extract, a 1 mL sub-sample was used to determine the total nematode abundance at low magnification, and then at higher magnification, 100 nematodes were identified to family-level [29,30]. Nematode abundances were expressed as numbers per 100 g of soil.

2.6. DNA Extraction

Freeze-dried soil samples were homogenised using a TissueLyser II (Qiagen, Clayton, VIC, Australia) at 30 Hz for 1.5 min. Freeze dried root samples were also homogenised

in a TissueLyser II (Qiagen) at 30 Hz, but for 3 min and with three sterile stainless steel screws (15 mm × 5 mm) to help breakup the tissue. Screws were sterilised in 1% bleach for five minutes and then rinsed with 0.001% DEPC water three times to remove any bleach. Soda-lime glass beads, that were used to assist in further tissue breakdown during DNA extractions were rinsed with 0.001% DEPC water to remove any residues and then sterilised in an autoclave.

DNA was extracted as previously described [31–33]. Briefly, 250 mg of powdered root (encompasses endo/ectorhizosphere and rhizoplane) or 5 g of powdered bulk soil was added to 1 mL (roots) or 10 mL (soil) of 0.12 M Na_2HPO_4 1% SDS solution. To assist with further tissue breakdown, root samples also had six glass beads and soil had 12 glass beads added to each tube. Both soil and root samples were then placed in a TissueLyser II (Qiagen) at 30 Hz for 1.5 min. To remove debris, the homogenate was then centrifuged at 1700 rcf for 5 min and the supernatant was added to an equal volume of phenol:chloroform:isoamyl alcohol (IAA) (25:24:1). Samples were centrifuged at 20,800 rcf for 5 min and chloroform:IAA (24:1) was added in equal volume to the aqueous phase to wash away the phenol. To precipitate DNA, isopropanol and 3 M sodium acetate was added to the aqueous phase (10:1:10) and centrifuged at 4 °C at 20,800 rcf for 30 min. The DNA pellet was washed with 70% ethanol and then resuspended in 50 µL of water. Resuspended DNA was then purified with a QIAquick Gel extraction kit (Qiagen) according to the manufacturer's instructions.

2.7. PCR Amplification and Sequencing of 16S and ITS rRNA Genes

Universal bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the primers 799F (5′- AAC MGG ATT AGA TAC CCK G G-3′) and 1193R (5′- ACG TCA TCC CCA CCT TCC-3′; Redford et al. [34]), each modified on the 5′ end to contain the Illumina overhang adapter for compatibility with the i5 and i7 Nextera XT indices, respectively. PCR reactions contained 2.5 µL DNA sample in 1X AmpliTaq Gold 360 master mix (Thermo Fisher, Scoresby, VIC, Australia) and 250 nM of each primer made to a total volume of 20 µL with molecular biology–grade water. Thermocycling conditions were as follows: 95 °C for 8 min; then 35 cycles of 95 °C for 20 s, 56 °C for 30 s, 72 °C for 45 s; followed by 72 °C for 7 min. Amplifications were performed using a SimpliAmp^{TM} 96-well Thermocycler (Applied Biosystems, Scoresby, VIC, Australia). Blank extraction controls and negative amplification controls were verified by gel electrophoresis. PCR products were purified using an 18% suspension of Sera-Mag Speed-beads Carboxyl Magnetic Beads (GE Healthcare, Rydalmere, NSW, Australia), added in a ratio of 1.8:1 vol PCR product.

A semi-nested PCR was used to amplify the ITS2 region as the conventional universal primers also amplify reads from *Musa* spp. [18]. We have previously shown that fungal community profiles generated using this nested PCR protocol are not significantly different from those generated using the conventional universal primers in a non-nested PCR [18]. The first PCR was performed using primers ITS1-F_KYO1 (5′-CTH GGT CAT TTA GAG GAA STA A-3′; Toju et al. [35]) and ITS4R (5′-TCC TCC GCT TAT TGA TAT GC-3′; White et al. [36]). Each reaction contained 2.5 µL of DNA sample in 1X AmpliTaq Gold 360 master mix (Thermo Fisher) and 250 nM of each primer made to a total volume of 20 µL with molecular biology–grade water. The product was then purified using magnetic beads [37] and used as the template for PCR 2, which targets the ITS2 region. The second PCR was performed using the primers gITS7F (5′-GTG ART CAT CGA RTC TTT G-3′; Ihrmark et al. [38]) and ITS4R [36] each modified on the 5′ end to contain the Illumina overhang adapter for compatibility with the P5 and i7 Nextera XT indices, respectively. Each reaction contained 4.5 µL of the purified product from the first PCR in 1X AmpliTaq Gold 360 master mix (Thermo Fisher) and 250 nM of each primer made to a total volume of 20 µL with molecular biology–grade water.

Thermocycling conditions of both PCR 1 and 2 were as follows: 95 °C for 8 min; then 15 cycles for PCR 1 or 25 cycles for PCR 2 of 95 °C for 20 s, 56 °C for 30 s, 72 °C for 1 min, followed by 72 °C for 7 min for both PCRs. Amplifications were performed using

a SimpliAmp^{TM} 96-well Thermocycler (Applied Biosystems). Blank extraction controls were verified by gel electrophoresis. PCR products were purified using an 18% suspension of Sera-Mag Speed-beads Carboxyl Magnetic Beads (GE Healthcare), added in a ratio of 1.8:1 vol PCR product. Indexed amplicons were then quantified using a PicoGreen dsDNA Quantification Kit (Invitrogen, Scoresby, VIC, Australia). Equal concentrations of each sample were pooled and sequenced on an Illumina MiSeq using 30% PhiX Control v3 (Illumina, Melbourne, VIC, Australia) and a MiSeq Reagent Kit v3 (600 cycles; Illumina) according to the manufacturer's instructions. All sequence data are available on the Sequence Read Archive (SRA) portal of NCBI under the accession number SAMN39959539.

2.8. Sequence Data Processing

Sequence data were processed using a modified UPARSE approach [39,40]. Briefly, forward reads were demultiplexed using cutadapt from QIIME2 (v2017.9.0; Martin et al. [41]; Bolyen et al. [42]) and quality filtered using USEARCH (v10.0.240; Edgar [43]). Reads were then mapped against representative sequences using fastx_uniques and cluster_otus (sequence similarity = 0.97) within USEARCH to generate OTU tables. Prior to OTU clustering, fungal ITS2 regions were extracted using ITSx (v1.0.11, Bengtsson-Palme et al. [44]) and chimeric sequences were removed using uchime2_ref of USEARCH against the UNITE 9.0 database [45]. Taxonomy was assigned to OTUs using BLASTN (v2.3.0+; Zhang et al. [46]) from QIIME2 against the SILVA 138 database for 16S rRNA genes [47] and UNITE 9.0 for fungal ITS2 genes. In addition, non-bacterial sequences were then removed from the 16S rRNA gene dataset using BIOM [48]. The remaining bacterial OTU sequences were then assigned Greengenes2 taxonomy (2022_10; McDonald et al. [49]) using BLASTN (v2.14.0). For selected bacterial OTUs the corresponding GTDB 214.1 [50] taxonomy strings for the best matches were obtained by entering the accession numbers or lowest taxonomy labels of the Greengenes2 [49] using the search tool within the GTDB website (https://gtdb.ecogenomic.org/, accessed on 29 February 2024; See Supplementary File). To facilitate generation of Weighted UniFrac distances [51], representative 16S OTU sequences were aligned using MAFFT (v7.221; Katoh and Standley [52]), masked using QIIME2, and then a midpoint-rooted phylogenetic tree was generated using Fast Tree (v2.1.9; Price et al. [53]). ITS2 samples were rarefied to 32,550 reads per sample and 16S samples were rarefied to 27,000 reads per sample (based on the samples with the lowest numbers of reads; Supplementary File S2). All alpha diversity metrics were produced using QIIME2 (detailed below).

2.9. Matching Sequences to the Common Core of Banana

The 16S and ITS2 sequences were compared to the sequences of the 36 core bacteria in Birt et al. (2022) [17] and the 21 core fungi in Birt et al. (2023) [18] using USEARCH [43]. Sequences that were $\geq$97% similar were considered adequate matches.

2.10. Statistical Analyses

The main and interactive effects of plant compartment (root and soil), and P treatment (control or elevated P) on univariate response variables (bacterial alpha diversity metrics (i.e., observed (Sobs) and predicted (Chao1) numbers of OTUs, as well as Shannon's, and Faith's Phylogenetic Diversity (PD) Indices [54–56]), enzyme activities, soil nematode abundance) were evaluated using ANOVA with Tukey's HSD post hoc comparisons. The main and interactive effects of plant compartment, and P rate on multivariate responses (bacterial, fungal, and nematode community composition) were assessed using permutational multivariate analysis of variance (PERMANOVA, Anderson [57]) as implemented in 'adonis2' function within the R package 'vegan' [58]. For these analyses, OTU tables were Hellinger transformed (square root of the relative abundances) and the distance method within 'adonis2' was set to Euclidean to preserve these input distances [59]. All analyses were performed using R (v4.0.5; R Core Team [60]). The only exceptions to this were the logistic regression models for plant performance data, which were constructed using the

step-wise selection process in Genstat V21 (VSNi). Bunch emergence and harvest data were analysed independently for each parameter and crop cycle.

3. Results

3.1. Soil Physicochemical Properties and Plant Performance

Relative to the control, P addition did not significantly influence soil physicochemical properties, except for an increase in Colwell P ($p < 0.01$; Table 1).

Table 1. Means and standard deviations for each soil physicochemical property in control (Ctrl) and elevated phosphorus (P) plots. Asterisks represent significant treatment effects as indicated by one-way ANOVA models ($p \leq 0.01$ **).

Soil Property	Ctrl	P	
pH	5.54 ± 0.24	5.5 ± 0.31	
Colwell P (mg/kg)	19.2 ± 1.92	56.4 ± 22.28	**
Phosphorus buffering index	218 ± 8.37	202 ± 14.83	
Total C (%)	1.70 ± 0.12	1.68 ± 0.19	
Labile C (mg/kg)	0.3 ± 0.07	0.3 ± 0.06	
Nitrate N (mg/kg)	3.08 ± 1.30	3.80 ± 1.61	
Organic matter (%)	2.16 ± 0.18	2.4 ± 0.21	
Organic carbon (%)	1.25 ± 0.10	1.40 ± 0.10	
Electrical conductivity (ds/m)	0.14 ± 0.01	0.14 ± 0.02	

In terms of plant responses, P addition was associated with significantly ($p < 0.001$) shorter vegetative cycles, relative to the controls (Table 2; Figure 1). This is evidenced by significantly faster bunch emergence and harvest in the first and second crop cycles, which are known as the plant crop and first ratoon, respectively (Table 2; Figure 1). In the plant crop, but not in the first ratoon, control plants had slightly heavier bunches (Table 2). Fruit yield, plant height, and foliar P, however, did not differ significantly between the control and elevated P plots, irrespective of the crop cycle (Table 2, Figures 1 and S4).

Table 2. Summary of agronomic data for plants in the first (plant) and second (ratoon 1) crop cycles grown in control (Ctrl) and elevated phosphorus (P) plots. For bunch emergence and harvest the values represent the number of days at which 50% or 85% of plants had emerged or harvested bunches, and the *p* values represent the difference between treatments (Ctrl vs. P) using logistic regression. For plant height, the values represent the mean heights with standard deviations when bunches were harvested, and the *p* values represent the difference between treatments (Ctrl vs. P) using ANOVA. For foliar P and bunch weight, the values represent means and standard deviations in each crop cycle, and the *p* values represent the differences between treatments (Ctrl vs. P) using ANOVA. For average fruit yield, the values represent the mean and standard deviation per year, and the *P* values represent the difference between treatments (Ctrl vs. P) using ANOVA ($p < 0.001$ ***; $p < 0.05$ *).

	Plant Crop				Ratoon 1			
Agronomic Variable	**Ctrl**	**P**	***p* Value**		**Ctrl**	**P**	***p* Value**	
Vegetative								
Bunch emergence time (d)								
50%	261	240	<0.001	***	524	484	<0.001	***
85%	302	276	<0.001	***	580	548	<0.001	***
Plant height (cm)	245 ± 5.7	231 ± 3.5	0.129		329 ± 3.2	326 ± 3.3	0.634	
Foliar P (mg g^{-1})	0.24 ± 0.035	0.25 ± 0.054	0.675		0.28 ± 0.078	0.24 ± 0.72	0.251	
Fruit								
Harvested bunches (d)								
50%	339	326	<0.001	***	641	610	<0.001	***
85%	366	354	<0.001	***	718	680	<0.001	***
Bunch weight (kg)	23.2 ± 0.71	20.1 ± 0.63	0.041	*	32.0 ± 0.88	30.8 ± 0.78	0.391	
Average Yield (kg $plant^{-1}$ yr^{-1})	21.5 ± 0.47	19.9 ± 0.47	0.078		28.8 ± 0.58	27.8 ± 0.62	0.372	

3.2. Potential Soil Microbial Enzyme Activities

P addition was associated with significant increases in the activities of alpha-glucosidase, chitinase, arylsulphatase, and acid phosphatase (Figure 2). No significant effects of P addition were observed for beta-glucosidase and xylosidase (Figure 2).

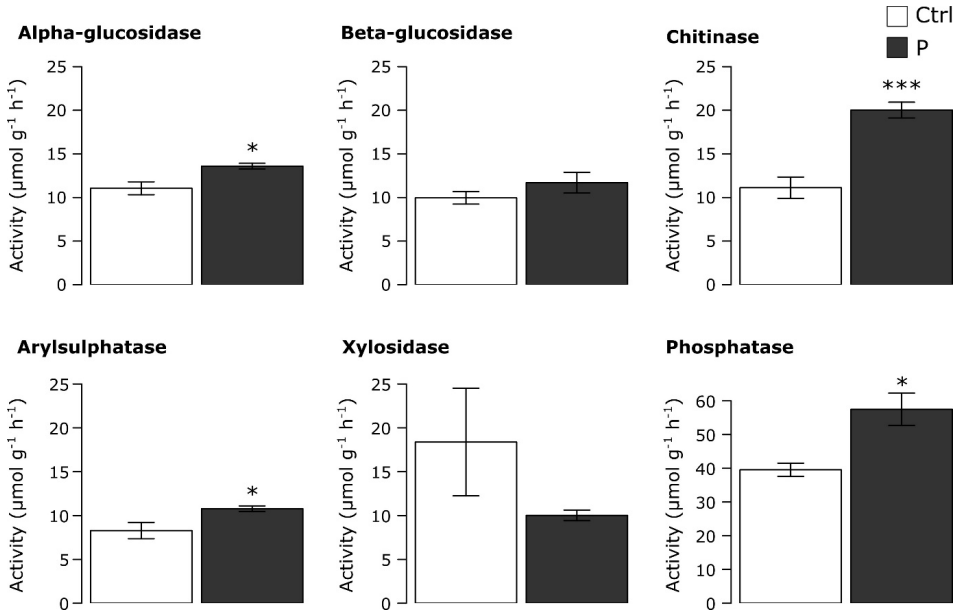

Figure 2. The potential activities of soil microbial enzymes in control (Ctrl) and elevated phosphorus (P) plots. Asterisks represent significant treatment effects as indicated by one-way ANOVA models ($p \leq 0.05$ *; $p \leq 0.001$ ***).

3.3. Effects of P Addition on the Diversity of Bacterial and Fungal Communities

The alpha diversity of bacterial and fungal communities was significantly larger in bulk soil than in roots, but was not significantly influenced by P addition, irrespective of compartment (Tables 3 and S1; Figures S1 and S2). Similarly, while the composition of bacterial and fungal communities (beta diversity) differed between compartments, significant shifts were not detected in response to P addition, irrespective of compartment (Table 4; Figure S3).

Table 3. ANOVA results summarising the main and interactive effects of compartment (soil and root) and treatment (control and elevated P) on the Shannon diversity of bacterial and fungal communities, and of treatment for nematode taxonomic groups in bulk soil. Asterisks indicate significant differences where: $p < 0.001$ ***; $p < 0.01$ **.

Target	Response Variable	Predictor Variable	*F* Value	*p* Value	
Bacteria	Shannon's Diversity Index	Compartment	11.63	0.004	**
		Treatment	0.02	0.900	
		Compartment: Treatment	0.10	0.751	
Fungi	Shannon's Diversity Index	Compartment	22.43	<0.001	***
		Treatment	0.10	0.761	
		Compartment: Treatment	0.08	0.785	
Nematodes	Shannon's Diversity Index	Treatment	2.33	0.165	

The bacterial community was dominated by Actinomycetota, Dormibacterota, Pseudomonadota, and single representatives from Bacillota and Chloroflexota. At the OTU level, roots harbored larger relative abundances of various Pseudomonadota such as Rhizobiales (OTU 7, 9, 12, and 20), Sphingomonadales (OTU 6), Burkholderiales (OTU 11, 16, 18, 31, and 43), Enterobacterales (OTU 1 and 5), and Pseudomonadales (OTU 2) (Figure 3). Bulk

soil had larger relative abundances of Actinomycetota (OTU 27 and 44), Bacillota (OTU 4), Chloroflexota (OTU 47), Dormibacterota (OTU 3, 46, and 1827), and some Pseudomonadota (OTU 13, 21, 26, and 28) (Figure 3).

Table 4. PERMANOVA results summarising the main and interactive effects of compartment (soil and root) and treatment (control and elevated P) on the composition of bacterial and fungal communities, and of treatment for the relative frequencies of different nematode taxonomic groups in bulk soil (counts per 100 g soil). Asterisks indicate significant differences where: $p < 0.001$ ***.

Target	Response Variable	Predictor Variable	*F* Value	R^2 Value	*p* Value	
Bacteria	OTU relative abundances (Hellinger transformed)	Compartment	14.90	46.0	<0.001	***
		Treatment	0.76	2.3	0.520	
		Compartment: Treatment	0.71	2.2	0.559	
	Weighted UniFrac distances	Compartment	25.13	58.8	<0.001	***
		Treatment	0.47	1.1	0.655	
		Compartment: Treatment	1.11	2.6	0.284	
Fungi	OTU relative abundances (Hellinger transformed)	Compartment	4.65	20.9	<0.001	***
		Treatment	0.99	4.4	0.387	
		Compartment: Treatment	0.66	2.9	0.941	
Nematodes	Taxon frequencies	Treatment	1.67	17.3	0.233	

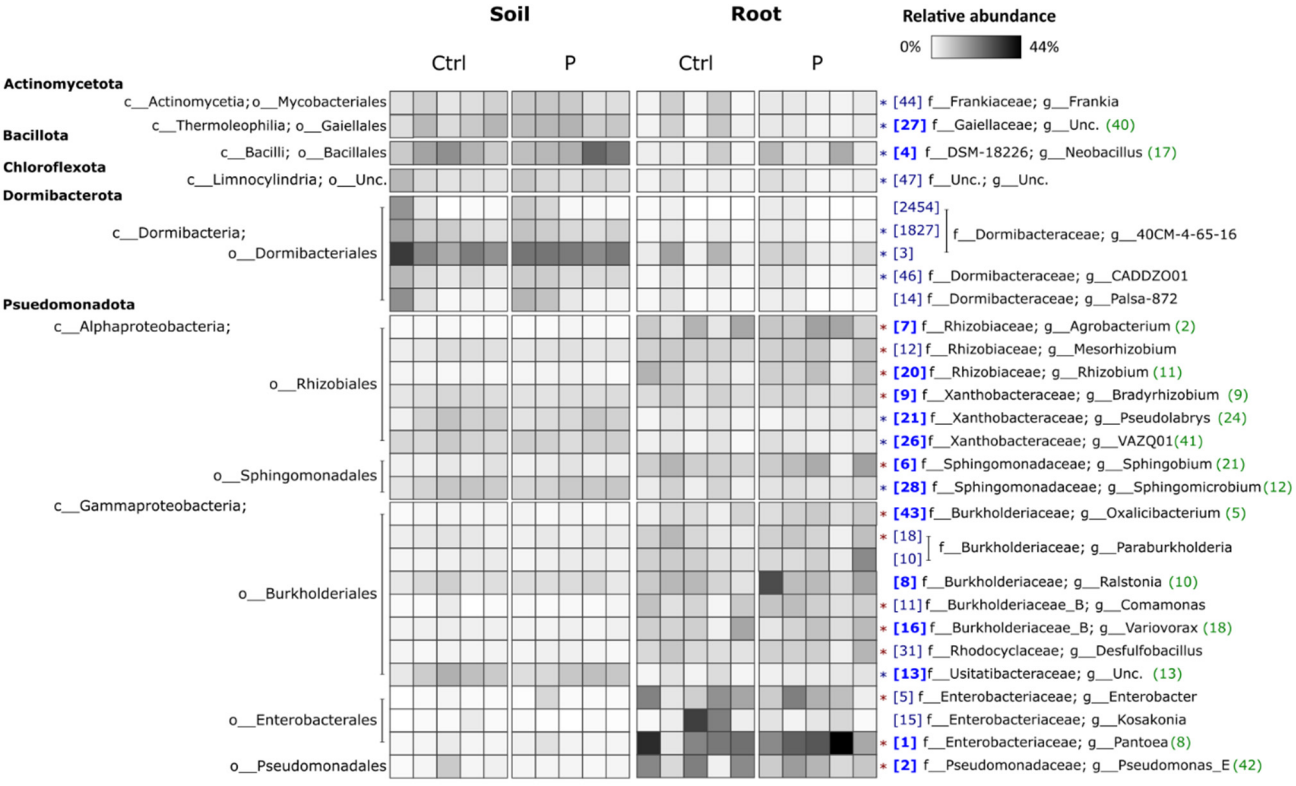

Figure 3. Heatmap summarising the composition of bulk soil and root-associated bacterial communities in control (Ctrl) and phosphorus-treated (P) plots. The OTUs listed are those present at ≥1.5% average relative abundance in any treatment. OTU identity numbers (OTU IDs) are shown in square brackets and are consistent between figures. The IDs of OTUs that have been identified as members of the bacterial core microbiome of banana are in bold, with the corresponding OTU ID number from Birt et al. [17] in green and in round brackets. The cell values are Hellinger transformed relative abundances. Asterisks represent OTUs that differed significantly between compartments (ANOVA; $p < 0.05$ *) with those in blue having larger relative abundances in bulk soil and those in red having larger relative abundances in roots.

Fungal communities were dominated by members of the Ascomycota, Basidiomycota, Mortierellomycota, and single representatives of Chytridiomycota and Mucoromycota. Roots were positively associated with Chaetosphariaceae (OTU 13), Hypocreales (OTU 3, 9, and 20), and an unclassified Sordariomycetes population (OTU 17) (Figure 4). Bulk soil harboured more Hypocreales (OTU 24), Sordariales (OTU 21), Trichosphaeriales (OTU 4 and 5), and Mortierellales (OTU 119) (Figure 4).

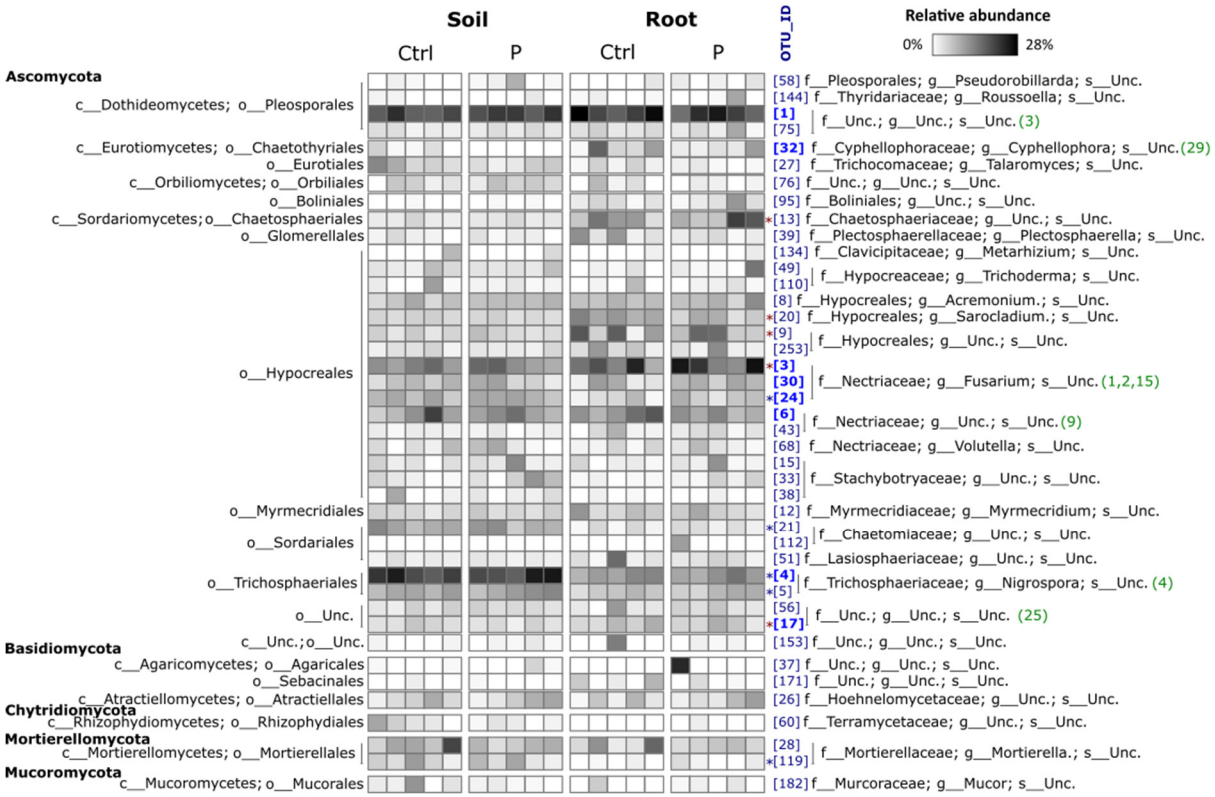

Figure 4. Heatmap summarising the composition of bulk soil and root-associated fungal communities in control (Ctrl) and phosphorus-treated (P) plots. The OTUs listed are those present at ≥2% average relative abundance in any treatment. OTU identity numbers (OTU IDs) are shown in square brackets and are consistent between figures. The IDs of OTUs that have been identified as members of the fungal core microbiome of banana are in bold, with the corresponding OTU ID number from Birt et al. [18] in green and in round brackets. The cell values are Hellinger transformed relative abundances. Asterisks represent OTUs that differed significantly between compartments (ANOVA; $p < 0.05$ *) with those in blue having larger relative abundances in bulk soil and those in red having larger relative abundances in roots.

3.4. Effects of P Addition on the Relative Abundance of Core Bacteria and Fungi

By comparing the 16S rRNA gene and ITS2 amplicon sequences in our study with those of the core *Musa* spp.–associated bacterial and fungal taxa reported by Birt et al. [17,18], we were able to detect 31 of 36 core bacteria and 18 of 21 core fungi (Tables S2 and S3). None of the bacterial OTUs matching core bacteria were significantly influenced by P addition, but most differed between compartments (Table S2). For fungi, OTU 10, a *Fusarium* population, significantly increased ($p = 0.019$) with P addition in roots (Figure 5). P addition alone did not significanlty influence any of the fungal OTUs identified as members of the core, and only a few differed between compartments (Table S3).

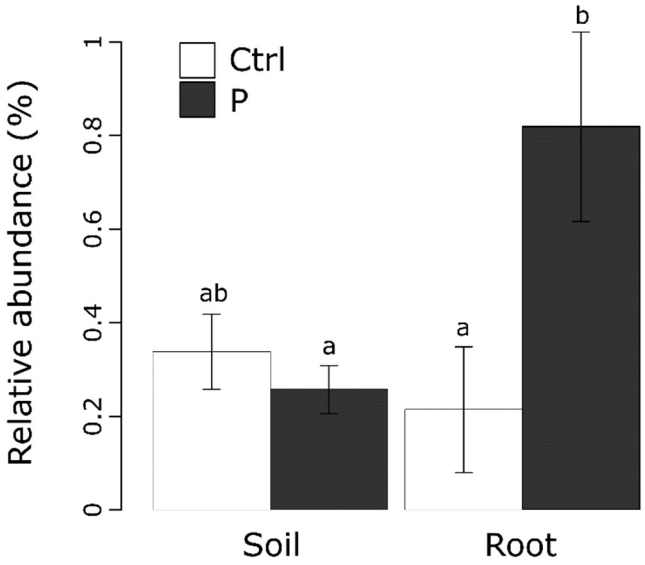

Figure 5. The relative abundance of a *Fusarium* (OTU 10) population between compartments in control (Ctrl) and elevated phosphorus (P) plots. Letters represent significant ($p = 0.019$) effects as indicated by Tukey post hoc analyses.

3.5. Nematode Numbers, Diversity, and Guild Representation

Total nematode counts did not differ significantly between P treatments (Table 5). Similarly, P addition did not significantly influence the alpha diversity of nematode communities in bulk soil (Tables 3 and S1). In terms of overall nematode community composition (Figure 6), PERMANOVA indicated that P addition had no significant effect on the abundances of different taxonomic groups (Table 4). Univariate ANOVA analyses, however, indicated that the representation of the Ca4 guild decreased in reponse to P addition (Table 5).

Table 5. ANOVA results summarising the effects of P application on the total numbers of nematodes in bulk soil (counts per 100 g soil), and each of the feeding groups and guilds. Asterisks indicate significant differences where: $p < 0.01$ **.

Response Variable	*F* Value	*p* Value	
Total nematodes	1.51	0.254	
Feeding groups			
Plant parasitic nematodes	1.7	0.231	
Fungivorous nematodes	2.29	0.169	
Bactivorous nematodes	0.01	0.925	
Predatory nematodes	1.48	0.259	
Omnivorous nematodes	3.34	0.105	
Guild			
Fu2	2.29	0.169	
Ba1	0.00	0.955	
Ba2	0.03	0.870	
Ba3	1.98	0.197	
Ca3	0.00	1.000	
Ca4	15.36	0.004	**
Om4	3.34	0.105	
pp2 ectoparasites	1.70	0.228	
pp3 endoparasites	1.00	0.347	

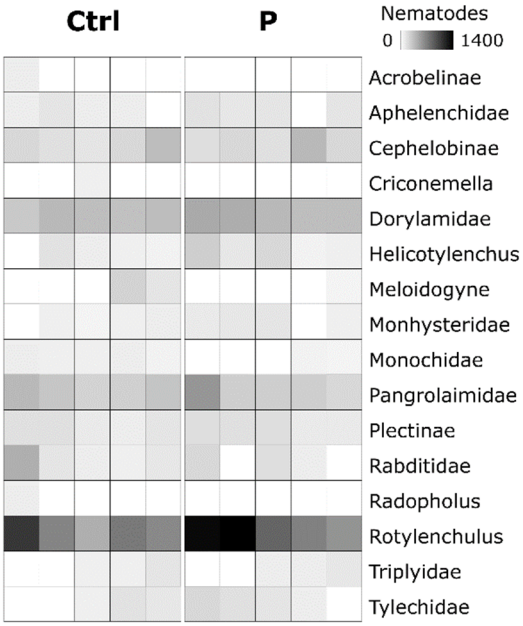

Figure 6. Heatmap summarising the composition of nematode communities in control (Ctrl) and phosphorus-treated (P) plots. Cell values represent individual counts per 100 g soil.

4. Discussion

4.1. P Addition Accelerated Crop Cycling but Did Not Impact Yield or Plant Height

As expected, the addition of phosphorus corresponded with an increase in soil Colwell P (Table 1). Since Colwell *p* values below 45 mg kg^{-1} are deemed insufficient for banana production [22], the rise from 19 mg kg^{-1} in the control plots to 56 mg kg^{-1} in the treatment plots marks a substantial enhancement for banana cultivation. Despite this, P addition had negligible effects on average yield, plant height, and foliar P (Figure 1; Table 2). The only significant effects of P addition were a shortening of the vegetative phase of plants in each crop cycle (i.e., earlier bunch emergence and harvest), and a slight reduction in bunch weight, albeit only in the plant crop (Figure 1, Table 2). Given the slower crop cycle of the control plants, their bunches had more time to grow. This may explain why they were slightly heavier when harvested. As yield and height did not significantly differ between treatments for the plant crop or ratoon one, our results indicate that P addition, at the moderate levels used here (40–50 kg P ha^{-1} y^{-1}), had negligible effects on the performance of banana plants. Our P applications are representative of those within recommended limits ($\leq$60 kg P ha^{-1} yr^{-1}) for banana farms in Queensland, which are intended to minimise nutrient pollution within Great Barrier Reef catchments [4,5].

4.2. Impacts of P on the Potential Activities of Soil Microbial Enzymes

The potential activities of xylosidase and beta-glucosidase were not significantly influenced by P addition (Figure 2). In contrast, P addition was associated with significant increases in the potential activities of alpha-glucosidase ($p < 0.05$), chitinase ($p < 0.001$), arylsulphatase ($p < 0.05$), and acid phosphatase ($p < 0.05$) (Figure 2). At a much larger P application rate (150 kg P ha^{-1} yr^{-1}) than used in our study, Wang et al. [61] observed a significant decrease in xylosidase activity; hence, the effect of P addition on xylosidase activity may depend on rate. Our finding that beta-glucosidase activity is not influenced by P addition is consistent with previous studies [61–63]. In contrast, while we observed a small but significant increase in alpha-glucosidase activity in response to P addition, Wang et al. [61] did not. For other enzymes our results are generally in agreement with previous studies indicating that P addition enhances chitinase, acid phosphatase [64], and arylsulphatase [65] activities, suggesting that P availability can influence microbial

activity [66]. At larger rates of P addition (>100 P kg ha^{-1} yr^{-1}) than tested in our study, P addition has also been observed to decrease acid phosphatase activity [8,62].

4.3. Bacterial and Fungal Diversity Was Not Influenced by P Addition

While P addition altered the potential activities of key nutrient cycling enzymes, these changes were not accompanied by changes in microbial diversity irrespective of compartment (i.e., bulk soil or roots). Thus, our results indicate a disconnect between microbial community structure and function. Nonetheless, this finding has been reported in many other studies and has been speculated to be associated with high levels of functional redundancy within the community [67–70]. In contrast with our findings, some studies have reported an association between available P and microbial diversity in banana plantations soils [15,16]. These associations, however, became apparent through exploratory analyses of soil chemical and microbial data derived from experiments unrelated to P manipulation. Consequently, while such studies may provide hypotheses to test, our data indicate that moderate P application rates are unlikely to strongly influence soil microbial diversity. Interestingly, however, at >100 kg P ha^{-1} yr^{-1}, but not smaller application rates, Pantigoso et al. [8] observed significant, and potentially deleterious, effects of P addition on the diversity of bacterial communities in the rhizosphere of blueberry. This suggests that further research is needed for banana production systems that use much larger P application rates than are typical in Australia.

Despite there being no effect of P on community composition, many bacterial OTUs and some fungal OTUs differed in relative abundance between bulk soil and roots (Figures 3, 4 and S3; Tables S2 and S3). As observed in previous studies, banana soil bacterial communities were dominated by members of the Acidobacteriota, Chloroflexota, Bacillota, and Pseudomonadota [15–17,71]. Roots harbored larger relative abundances of various Pseudomonadota populations, whereas representatives of the Acidobacteriota, Chloroflexota and Bacillota were relatively more prominent in bulk soils (Figure 3). Fungal communites were dominated by members of the Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, and Mucoromycota, which is in agreement with other studies of banana soils [18,71]. Only OTUs within the Ascomycota and Mortierellomycota differed between roots and bulk soils (Figure 4). Despite reports of arbuscular mycorrhizae in *Musa* spp. [10], the minimum, mean, and maximum relative abundance of all Glomeromycota OTUs combined was 0%, 0.04%, and 0.18%, respectively. Some research indicates that host plants mediate the impacts of fertilisation on arbuscular mycorrhiza populations [72]; however, further research with more targeted primers may provide different results for these taxa [73].

4.4. The Core Microbiome Is Not Effectively Manipulated by P

Previously, we have identified a core bacterial [17] and core fungal microbiome of *Musa* spp. [18]. By comparing the 16S rRNA gene and ITS2 sequences from this study to representatives of the core microbiome, we were able to detect 31 of the 36 core bacteria and 18 of the 21 core fungi (Tables S2 and S3). The five core bacteria that we did not observe in our study were reported to be exclusive to banana leaves [17], which likely explains why we did not detect them in bulk soil or roots. Additionally, two of the three core fungal populations that we did not detect were reported to be exclusive to the pseudostem or leaves of banana [18]. With this taken into consideration, in this study, we observed 100% of the core bacterial and 95% of the core fungal soil and root microbiome, strengthening the hypothesis that these organisms are associated with *Musa* spp.

P addition did not influence the relative abundances of bacterial or fungal OTUs identified as members of the core microbiome, with the exception of fungal OTU 10, a *Fusarium* population, which significantly increased ($p = 0.019$) in roots (Figure 4; Table S3). Importantly, not all *Fusarium* populations are pathogentic. Many *Fusarium* populations are associated with healthy plants [74], demonstrate the ability to suppress the growth of pathogenic strains [75–77], and are now recognised as key members of the core mycobiome

of banana [18]. To our knowledge, no studies have explored the effects of P addition at different rates on *Fusarium* populations, both pathogenic and commensal, and how this influences their ability to colonise the root of plants. Given that OTU 10 has been identified as a potential core fungal OTU of banana, further investigation into its ability to suppress disease occurance and the potential of P addition as a fungal disease management tool is warranted. Conversely, as there were no significant effects of P addition on the overall composition of bacterial communities, moderate levels of P application are unlikely to be an effective management tool to manipulate the core bacterial microbiome of banana.

4.5. Nematodes Are Not Affected by the Addition of P

P fertilisation did not cause a change in the total abundance of nematodes except for Guild 4 carnivores (Ca4) (Table 5). P addition has been shown to increase [78], decrease [79], or, as we observed, have no effect on total nematode abundace [11]. Likewise, predatory nematodes have been observed to have lower density than other trophic groups [80,81] and become less frequent in response to P addition [82]. While carnivorous nematodes prey on plant parasitic nematodes and other micro-organisms, there were no changes in the abundances of these prey groups (Tables 2, 3 and 5). This can likely be attributed to the functional redundancy often observed in nematode communities, where multiple guilds mediate similar ecosystem functions [83]. As we see no effect of P addition on total predatory and Ca3 carnivorous nematodes, it is likely that Ca3 nematodes were serving a similar ecosystem function to the Ca4 guild, and thus no disturbance was observed. Additionally, as P application did not have a significant effect on bacterial, fungal, or nematode communities, this indicates that moderate P addition over two years had negligible effects on multiple trophic groups in banana plantation soils.

5. Conclusions

Our study indicates that the addition of P fertiliser within recommended limits ($\leq$60 kg P ha^{-1} yr^{-1}) for Queensland banana plantations can increase the potential activities of nutrient-cycling enzymes but is unlikely to have strong impacts on microbial diversity. Within our system, this moderate level of P addition was sufficient to elicit a significant increase in P availability. Nonetheless, P application rates in other banana production areas around the world often exceed these rates; hence, our findings may be less representative of those systems. It is important to acknowledge, however, that a change in microbial community structure is not always a desirable outcome, and could indicate dybiosis caused by P addtion. For example, the only effect of P addition that we observed on microbial community structure was a small increase in a *Fusarium* population in roots, which considering that this genus includes a key pathogen of banana, may not be favourable for production. Phosphorus addition increased the potential activity of enzymes involed in carbon, nitrogen, sulphur, and phosphorus nutrient cycling, potentially leading to better mobilisation of these nutrients in the soil. While we have investigated the effects of P on banana microbiomes within the Australian industry's most common soil type [84], we acknowledge that our results may vary in other soils. Our findings also reflect minimal overall differences between bananas grown with and without added P over two years. It is possible that larger effects would be apparent at different stages throughout this time and after a longer duration of imposed management. It is also important to acknowledge that the effects of P addition on microbial communities may be direct, or indirect via impacts on the host. Further research into the impacts of P addition on banana farms should look at the impacts of P on microbiomes and core taxa when applied at different rates and frequencies across a wider range of edaphic conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applmicrobiol4040108/s1, Clarke et al. Supplementary information—a zip file containing: (1) Clarke et al. Supplementary File S1—OTUs in heatmap.csv (information about the OTUs in the heatmap); (2) Clarke et al. Supplementary File S2—Sequencing stats.csv (details of the

sequencing run); and (3) Clarke et al. Supplementary information.docx (contains Tables S1–S3 and Figures S1–S4).

Author Contributions: A.B.P. and P.G.D. secured funding. H.R.L.-G. collected samples and performed biochemical tests. A.B.P. characterised nematodes. S.I.-B. designed and managed the field experiment. A.-B.C.C. and R.L. performed marker gene sequencing. P.G.D., A.-B.C.C., H.R.L.-G. and J.S. analysed the data. A.-B.C.C., H.R.L.-G. and P.G.D. wrote the paper with input from all authors. All authors have read and agreed to the published version of the manuscript.

Funding: The microbiome and nematology components of the work were funded by the Australian Centre for International Agricultural Research (ACIAR HORT/2018/192). Crop establishment and agronomy were components of 'The Banana Nutrient Rate Trials', project RP191, funded by the Queensland Reef Water Quality Program, Queensland Government Department of Environment and Science.

Data Availability Statement: The raw sequence data associated with this study are available through the NCBI under BioProject PRJNA1077075 accession number SAMN39959539. The representative sequences OTUs shown in the heatmaps, discussed in the text, or identified as members of the core microbiome are provided in the online Supplementary Material.

Acknowledgments: The late Phil Moody is acknowledged for his contribution to ensuring that the phosphorus investigation was undertaken, and Alex Lindsay is acknowledged for his agronomic advice and interpretations relative to crop husbandry. We gratefully acknowledge Rebecca Murray, Curtis Lanham, and the agronomy team at the Centre for Wet Tropics Agriculture, South Johnstone, for technical assistance.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. FAO. Bananas: Commodity in Focus. Available online: https://www.fao.org/markets-and-trade/commodities/bananas/en/ (accessed on 25 July 2023).
2. Aurore, G.; Parfait, B.; Fahrasmane, L. Bananas, raw materials for making processed food products. *Trends Food Sci. Tech.* **2009**, *20*, 78–91. [CrossRef]
3. Lahav, E. Banana nutrition. In *Bananas and Plantains*; Gowen, S., Ed.; Springer: Dordrecht, The Netherlands, 1995; pp. 258–316. ISBN 978-94-011-0737-2.
4. Department of Environment and Science. *Prescribed Methodology for Banana Cultivation*; Reef Water Quality Program; Queensland Government: Brisbane, QLD, Australia, 2022.
5. Queensland Government. Requirements for Banana Growers. Reef Protection Regulations. Available online: https://www.qld.gov.au/environment/agriculture/sustainable-farming/reef/reef-regulations/bananas (accessed on 7 March 2024).
6. Dai, Z.; Liu, G.; Chen, H.; Chen, C.; Wang, J.; Ai, S.; Wei, D.; Li, D.; Ma, B.; Tang, C.; et al. Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. *ISME J.* **2020**, *14*, 757–770. [CrossRef]
7. McKnight, M.M.; Qu, Z.; Copeland, J.K.; Guttman, D.S.; Walker, V.K. A practical assessment of nano-phosphate on soybean (Glycine max) growth and microbiome establishment. *Sci. Rep.* **2020**, *10*, 9151. [CrossRef] [PubMed]
8. Pantigoso, H.A.; Manter, D.K.; Vivanco, J.M. Phosphorus addition shifts the microbial community in the rhizosphere of blueberry (Vaccinium corymbosum L.). *Rhizosphere* **2018**, *7*, 1–7. [CrossRef]
9. Xiao, D.; Che, R.; Liu, X.; Tan, Y.; Yang, R.; Zhang, W.; He, X.; Xu, Z.; Wang, K. Arbuscular mycorrhizal fungi abundance was sensitive to nitrogen addition but diversity was sensitive to phosphorus addition in karst ecosystems. *Biol. Fertil. Soils* **2019**, *55*, 457–469. [CrossRef]
10. Declerck, S.; Plenchette, C.; Strullu, D.G. Mycorrhizal dependency of banana (Musa acuminata, AAA group) cultivar. *Plant Soil* **1995**, *176*, 183–187. [CrossRef]
11. Olatunji, O.A.; Gong, S.; Tariq, A.; Pan, K.; Sun, X.; Chen, W.; Zhang, L.; Dakhil, M.A.; Huang, D.; Tan, X. The effect of phosphorus addition, soil moisture, and plant type on soil nematode abundance and community composition. *J. Soils Sediments* **2019**, *19*, 1139–1150. [CrossRef]
12. Jiang, N.; Wei, K.; Pu, J.; Zhang, Y.; Xie, H.; Bao, H.; Chen, L. Effects of the reduction in chemical fertilizers on soil phosphatases encoding genes (phoD and phoX) under crop residue mulching. *Appl. Soil Ecol.* **2022**, *175*, 104428. [CrossRef]
13. Neal, A.; McLaren, T.; Lourenço Campolino, M.; Hughes, D.; Marcos Coelho, A.; Gomes de Paula Lana, U.; Aparecida Gomes, E.; Morais de Sousa, S. Crop type exerts greater influence upon rhizosphere phosphohydrolase gene abundance and phylogenetic diversity than phosphorus fertilization. *FEMS Microbiol. Ecol.* **2021**, *97*, fiab033. [CrossRef]
14. Wang, L.; Wen, Y.; Tong, R.; Zhang, H.; Chen, H.; Hu, T.; Liu, G.; Wang, J.; Zhu, L.; Wu, T. Understanding Responses of Soil Microbiome to the Nitrogen and Phosphorus Addition in Metasequoia glyptostroboides Plantations of Different Ages. *Microb. Ecol.* **2022**, *84*, 565–579. [CrossRef]

15. Fan, P.; Lai, C.; Yang, J.; Hong, S.; Yang, Y.; Wang, Q.; Wang, B.; Zhang, R.; Jia, Z.; Zhao, Y.; et al. Crop rotation suppresses soil-borne Fusarium wilt of banana and alters microbial communities. *Arch. Agron. Soil Sci.* **2022**, *68*, 447–459. [CrossRef]
16. Shen, Z.; Ruan, Y.; Xue, C.; Zhong, S.; Li, R.; Shen, Q. Soils naturally suppressive to banana Fusarium wilt disease harbor unique bacterial communities. *Plant Soil* **2015**, *393*, 21–33. [CrossRef]
17. Birt, H.W.G.; Pattison, A.B.; Skarshewski, A.; Daniells, J.; Raghavendra, A.; Dennis, P.G. The core bacterial microbiome of banana (Musa spp.). *Environ. Microbiome* **2022**, *17*, 46. [CrossRef] [PubMed]
18. Birt, H.W.G.; Pattison, A.B.; Skarshewski, A.; Daniells, J.; Raghavendra, A.; Dennis, P.G. The core fungal microbiome of banana (Musa spp.). *Front. Microbiol.* **2023**, *14*, 1127779. [CrossRef] [PubMed]
19. Cannon, M.G.; Smith, C.D.; Murtha, G.G. *Soils of the Cardwell-Tully Area, North Queensland*; Division of Soils Divisional Report; CSIRO: Canberra, ACT, Australia, 1992; ISBN 0-643-05081-7.
20. Armour, J.D.; Nelson, P.N.; Daniells, J.W.; Rasiah, V.; Inman-Bamber, N.G. Nitrogen leaching from the root zone of sugarcane and bananas in the humid tropics of Australia. *Agric. Ecosyst. Environ.* **2013**, *180*, 68–78. [CrossRef]
21. Kernot, I.; Lindsay, S. *Tropical Banana Information Kit*; Agrilink: Your growing guide to better farming series; Queensland Department of Primary Industries: Brisbane, QLD, Australia, 1998; ISBN 978-0-7242-6725-5.
22. Armour, J. *Nutrient Management Plan for the Banana Industry (of North Queensland)*; Australian Banana Growers' Council: Rocklea, QLD, Australia, 2018.
23. Rayment, G.E.; Lyons, D.J. *Soil Chemical Methods—Australasia*; CSIRO Publishing: Melbourne, VIC, Australia, 2011; ISBN 0-643-06768-X.
24. Moody, P.; Cong, P. *Soil Constraints and Management Package (SCAMP): Guidelines for Management of Tropical Upland Soils*; Australian Centre for International Agricultural Research: Canberra, ACT, Australia, 2008.
25. Edwards, T. What is Soil Organic Carbon? Available online: https://www.agric.wa.gov.au/measuring-and-assessing-soils/what-soil-organic-carbon (accessed on 23 February 2024).
26. Lapis-Gaza, H.R.; Pattison, A.B. Functional Soil Biological Measurements to Support Healthy Soils. In *The Plant Microbiome: Methods and Protocols*; Carvalhais, L.C., Dennis, P.G., Eds.; Springer: New York, NY, USA, 2021; pp. 265–281, ISBN 978-1-07-161040-4.
27. Zarcinas, B.A.; Cartwright, B.; Spouncer, L.R. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil Sci. Plant Anal.* **1987**, *18*, 131–146. [CrossRef]
28. Whiteheads, A.G.; Hemming, J.R. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. Appl. Biol.* **1965**, *55*, 25–38. [CrossRef]
29. Goodey, T.; Goodey, J.B. *Soil and Freshwater Nematodes*, 2nd ed.; Butler & Tanner Ltd.: London, UK, 1963.
30. Yeates, G.W.; Bongers, T.; de Goede, R.G.M.; Freckman, D.W.; Georgieva, S.S. Feeding habits in soil nematode families and genera—An outline for soil ecologists. *J. Nematol.* **1993**, *25*, 315–331. [PubMed]
31. Chan, C.M.; Lyons, R.; Dennis, P.G.; Lant, P.; Pratt, S.; Laycock, B. Effect of Toxic Phthalate-Based Plasticizer on the Biodegradability of Polyhydroxyalkanoate. *Environ. Sci. Technol.* **2022**, *56*, 17732–17742. [CrossRef]
32. Deng, H.; Zhang, B.; Yin, R.; Wang, H.; Mitchell, S.M.; Griffiths, B.S.; Daniell, T.J. Long-term effect of re-vegetation on the microbial community of a severely eroded soil in sub-tropical China. *Plant Soil* **2010**, *328*, 447–458. [CrossRef]
33. Pennanen, T.; Caul, S.; Daniell, T.J.; Griffiths, B.S.; Ritz, K.; Wheatley, R.E. Community-level responses of metabolically-active soil microorganisms to the quantity and quality of substrate inputs. *Soil Biol. Biochem.* **2004**, *36*, 841–848. [CrossRef]
34. Redford, A.J.; Bowers, R.M.; Knight, R.; Linhart, Y.; Fierer, N. ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environ. Microbiol.* **2010**, *12*, 2885–2893. [CrossRef]
35. Toju, H.; Tanabe, A.S.; Yamamoto, S.; Sato, H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS ONE* **2012**, *7*, e40863. [CrossRef] [PubMed]
36. White, T.; Bruns, T.; Lee, S.; Taylor, J.; Innis, M.; Gelfand, D.; Sninsky, J. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In *Pcr Protocols: A Guide to Methods and Applications*; Academic Press: Cambridge, MA, USA, 1990; Volume 31, pp. 315–322.
37. Rohland, N.; Reich, D. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Res.* **2012**, *22*, 939–946. [CrossRef] [PubMed]
38. Ihrmark, K.; Bödeker, I.T.M.; Cruz-Martinez, K.; Friberg, H.; Kubartova, A.; Schenck, J.; Strid, Y.; Stenlid, J.; Brandström-Durling, M.; Clemmensen, K.E.; et al. New primers to amplify the fungal ITS2 region—Evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol. Ecol.* **2012**, *82*, 666–677. [CrossRef] [PubMed]
39. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef]
40. Forstner, C.; Orton, T.G.; Skarshewski, A.; Wang, P.; Kopittke, P.M.; Dennis, P.G. Effects of graphene oxide and graphite on soil bacterial and fungal diversity. *Sci. Total Environ.* **2019**, *671*, 140–148. [CrossRef] [PubMed]
41. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J.* **2011**, *17*, 10–12. [CrossRef]
42. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef] [PubMed]
43. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460–2461. [CrossRef] [PubMed]

44. Bengtsson-Palme, J.; Ryberg, M.; Hartmann, M.; Branco, S.; Wang, Z.; Godhe, A.; De Wit, P.; Sánchez-García, M.; Ebersberger, I.; de Sousa, F.; et al. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods Ecol. Evol.* **2013**, *4*, 914–919. [CrossRef]
45. Abarenkov, K.; Nilsson, R.H.; Larsson, K.-H.; Taylor, A.F.S.; May, T.W.; Frøslev, T.G.; Pawlowska, J.; Lindahl, B.; Põldmaa, K.; Truong, C.; et al. The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: Sequences, taxa and classifications reconsidered. *Nucleic Acids Res.* **2024**, *52*, D791–D797. [CrossRef]
46. Zhang, Z.; Schwartz, S.; Wagner, L.; Miller, W. A Greedy Algorithm for Aligning DNA Sequences. *J. Comput. Biol.* **2000**, *7*, 203–214. [CrossRef] [PubMed]
47. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2012**, *41*, D590–D596. [CrossRef]
48. McDonald, D.; Clemente, J.C.; Kuczynski, J.; Rideout, J.R.; Stombaugh, J.; Wendel, D.; Wilke, A.; Huse, S.; Hufnagle, J.; Meyer, F. The Biological Observation Matrix (BIOM) format or: How I learned to stop worrying and love the ome-ome. *Gigascience* **2012**, *1*, 1–6. [CrossRef] [PubMed]
49. McDonald, D.; Jiang, Y.; Balaban, M.; Cantrell, K.; Zhu, Q.; Gonzalez, A.; Morton, J.T.; Nicolaou, G.; Parks, D.H.; Karst, S.M.; et al. Greengenes2 unifies microbial data in a single reference tree. *Nat. Biotechnol.* **2023**, *42*, 715–718. [CrossRef]
50. Chaumeil, P.-A.; Mussig, A.J.; Hugenholtz, P.; Parks, D.H. GTDB-Tk: A toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* **2020**, *36*, 1925–1927. [CrossRef] [PubMed]
51. Lozupone, C.; Knight, R. UniFrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **2005**, *71*, 8228–8235. [CrossRef] [PubMed]
52. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef] [PubMed]
53. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS ONE* **2010**, *5*, e9490. [CrossRef] [PubMed]
54. Shannon, C.E. A Mathematical Theory of Communication. *Bell Syst. Tech. J.* **1948**, *27*, 379–423. [CrossRef]
55. Chao, A. Nonparametric Estimation of the Number of Classes in a Population. *Scand. J. Stat.* **1984**, *11*, 265–270.
56. Faith, D.P. Conservation evaluation and phylogenetic diversity. *Biol. Conserv.* **1992**, *61*, 1–10. [CrossRef]
57. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **2001**, *26*, 32–46. [CrossRef]
58. Oksanen, J.; Simpson, G.L.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; O'Hara, R.B.; Solymos, P.; Stevens, M.H.H.; Szoecs, E.; et al. Vegan: Community Ecology Package. 2022. Available online: https://cran.r-project.org/web/packages/vegan/index.html (accessed on 28 August 2024).
59. Legendre, P.; Gallagher, E.D. Ecologically meaningful transformations for ordination of species data. *Oecologia* **2001**, *129*, 271–280. [CrossRef] [PubMed]
60. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Stastistical Computing: Vienna, Austria, 2021.
61. Wang, C.; Mori, T.; Mao, Q.; Zhou, K.; Wang, Z.; Zhang, Y.; Mo, H.; Lu, X.; Mo, J. Long-term phosphorus addition downregulates microbial investments on enzyme productions in a mature tropical forest. *J. Soils Sediments* **2020**, *20*, 921–930. [CrossRef]
62. Zheng, M.; Huang, J.; Chen, H.; Wang, H.; Mo, J. Responses of soil acid phosphatase and beta-glucosidase to nitrogen and phosphorus addition in two subtropical forests in southern China. *Eur. J. Soil Biol.* **2015**, *68*, 77–84. [CrossRef]
63. Luo, R.; Fan, J.; Wang, W.; Luo, J.; Kuzyakov, Y.; He, J.-S.; Chu, H.; Ding, W. Nitrogen and phosphorus enrichment accelerates soil organic carbon loss in alpine grassland on the Qinghai-Tibetan Plateau. *Sci. Total. Environ.* **2019**, *650*, 303–312. [CrossRef] [PubMed]
64. Liu, M.; Gan, B.; Li, Q.; Xiao, W.; Song, X. Effects of Nitrogen and Phosphorus Addition on Soil Extracellular Enzyme Activity and Stoichiometry in Chinese Fir (Cunninghamia lanceolata) Forests. *Front. Plant Sci.* **2022**, *13*, 834184. [CrossRef] [PubMed]
65. Wang, S.; Zhou, K.; Mori, T.; Mo, J.; Zhang, W. Effects of phosphorus and nitrogen fertilization on soil arylsulfatase activity and sulfur availability of two tropical plantations in southern China. *Forest Ecol. Manag.* **2019**, *453*, 117613. [CrossRef]
66. Zhang, Y.; Wang, C.; Li, Y. Contrasting effects of nitrogen and phosphorus additions on soil nitrous oxide fluxes and enzyme activities in an alpine wetland of the Tibetan Plateau. *PLoS ONE* **2019**, *14*, e0216244. [CrossRef]
67. Burke, C.; Steinberg, P.; Rusch, D.; Kjelleberg, S.; Thomas, T. Bacterial community assembly based on functional genes rather than species. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 14288–14293. [CrossRef] [PubMed]
68. Frossard, A.; Gerull, L.; Mutz, M.; Gessner, M.O. Disconnect of microbial structure and function: Enzyme activities and bacterial communities in nascent stream corridors. *ISME J.* **2012**, *6*, 680–691. [CrossRef] [PubMed]
69. LeBrun, E.S.; King, R.S.; Back, J.A.; Kang, S. Microbial Community Structure and Function Decoupling Across a Phosphorus Gradient in Streams. *Microb. Ecol.* **2018**, *75*, 64–73. [CrossRef] [PubMed]
70. Szejgis, J.; Carrillo, Y.; Jeffries, T.C.; Dijkstra, F.A.; Chieppa, J.; Horn, S.; Bristol, D.; Maisnam, P.; Eldridge, D.; Nielsen, U.N. Altered rainfall greatly affects enzyme activity but has limited effect on microbial biomass in Australian dryland soils. *Soil Biol. Biochem.* **2024**, *189*, 109277. [CrossRef]
71. Cabanás, C.G.-L.; Fernández-González, A.J.; Cardoni, M.; Valverde-Corredor, A.; López-Cepero, J.; Fernández-López, M.; Mercado-Blanco, J. The banana root endophytome: Differences between mother plants and suckers and evaluation of selected bacteria to control *fusarium oxysporum* f.sp. cubense. *J. Fungi* **2021**, *7*, 194. [CrossRef]

72. Liu, Y.; Shi, G.; Mao, L.; Cheng, G.; Jiang, S.; Ma, X.; An, L.; Du, G.; Johnson, N.C.; Feng, H. Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. *New Phytol.* **2012**, *194*, 523–535. [CrossRef] [PubMed]
73. Krüger, M.; Stockinger, H.; Krüger, C.; Schüßler, A. DNA-based species level detection of Glomeromycota: One PCR primer set for all arbuscular mycorrhizal fungi. *New Phytol.* **2009**, *183*, 212–223. [CrossRef] [PubMed]
74. Nel, B.; Steinberg, C.; Labuschagne, N.; Viljoen, A. Isolation and characterization of nonpathogenic Fusarium oxysporum isolates from the rhizosphere of healthy banana plants. *Plant Pathol.* **2006**, *55*, 207–216. [CrossRef]
75. Forsyth, L.M.; Smith, L.J.; Aitken, E.A.B. Identification and characterization of non-pathogenic Fusarium oxysporum capable of increasing and decreasing Fusarium wilt severity. *Mycol. Res.* **2006**, *110*, 929–935. [CrossRef]
76. Nel, B.; Steinberg, C.; Labuschagne, N.; Viljoen, A. The potential of nonpathogenic Fusarium oxysporum and other biological control organisms for suppressing fusarium wilt of banana. *Plant Pathol.* **2006**, *55*, 217–223. [CrossRef]
77. Belgrove, A.; Steinberg, C.; Viljoen, A. Evaluation of Nonpathogenic Fusarium oxysporum and Pseudomonas fluorescens for Panama Disease Control. *Plant Dis.* **2011**, *95*, 951–959. [CrossRef]
78. Sun, F.; Tariq, A.; Chen, H.; He, Q.; Guan, Y.; Pan, K.; Chen, S.; Li, J.; Zhao, C.; Wang, H.; et al. Effect of nitrogen and phosphorus application on agricultural soil food webs. *Arch. Agron. Soil. Sci.* **2017**, *63*, 1176–1186. [CrossRef]
79. Qi, Y.; Sun, X.; Peng, S.; Tan, X.; Zhou, S. Effects of fertilization on soil nematode communities in an alpine meadow of Qinghai-Tibet plateau. *Front. Ecol. Evol.* **2023**, *11*, 1122505. [CrossRef]
80. Todd, T.C. Effects of management practices on nematode community structure in tallgrass prairie. *Appl. Soil. Ecol.* **1996**, *3*, 235–246. [CrossRef]
81. Zhong, S.; He, Y.; Zeng, H.; Mo, Y.; Jin, Z. Effects of banana wilt disease on soil nematode community structure and diversity. *Afr. J. Biotechnol.* **2011**, *10*, 12759–12767. [CrossRef]
82. Nielsen, U.N.; Prior, S.; Delroy, B.; Walker, J.K.M.; Ellsworth, D.S.; Powell, J.R. Response of belowground communities to short-term phosphorus addition in a phosphorus-limited woodland. *Plant Soil* **2015**, *391*, 321–331. [CrossRef]
83. Ferris, H. Nematodes and the soil food web: Understanding healthy soils. In Proceedings of the California Conference on Biological Control IV, Berkeley, CA, USA, 13–15 July 2004.
84. Orr, R.; Northfield, T.D.; Pattison, A.; Nelson, P.N. Soil physicochemical characteristics and leaf nutrient contents on banana farms of North Queensland, Australia. *Crop Pasture Sci.* **2023**, *74*, 483–493. [CrossRef]