Plant Soil (2025) 514:1923–1940
https://doi.org/10.1007/s11104-025-07493-5

RESEARCH ARTICLE

Wheat growth and phosphorus uptake from polyculture algal biofilms are synergistically modulated by arbuscular mycorrhizal fungi and *Serendipita vermifera*

Xinyu Gan · Jennifer Janus · Sabine Willbold · Vitalij Dombinov · Arnd J. Kuhn · Wulf Amelung · Diana Reinecke · Dean Calahan · Ladislav Nedbal · Holger Klose · Silvia D. Schrey

Received: 26 February 2025 / Accepted: 20 April 2025 / Published online: 7 May 2025

Abstract
Background and aims Phosphorus (P) from surface waters can be captured in algal biomass, which can be used as a fertilizer. We investigated the efficiency of polyculture algal biofilms produced on municipal wastewater effluent as a P fertilizer for wheat. We asked whether arbuscular mycorrhizal fungi (AMF) and the beneficial root endophyte *Serendipita vermifera* influence plant performance and P uptake.
Methods Two pot experiments were performed with wheat fertilized with algal biofilms or highly available triple superphosphate (TSP) at a rate of 37 mg P kg^{-1}, corresponding to 56.8 kg ha^{-1}. In the second experiment, plants were inoculated with AMF (*Rhizoglomus irregulare, Funneliformis mosseae, F. geosporum), S. vermifera*, or both. P species contained in the algal biofilm and P release dynamics were analyzed by liquid-state ^{31}P nuclear magnetic resonance spectrometry and leachate analyses.
Results Algal biofilms contained high levels of orthophosphate with low water solubility. P recovery by wheat was lower than from TSP, as indicated by plant total dry matter and total P. In algae-fertilized wheat, AMF reduced growth but not P uptake, while *S. vermifera* in dual inoculation with AMF mitigated the adverse effects. *S. vermifera* significantly increased root growth and P content in roots when co-inoculated with AMF.
Conclusion Polyculture algal biomass is an effective, less leaching-prone organic P source for wheat. The synergistic effect of *S. vermifera* as a root

Responsible Editor: Peter Christie.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-025-07493-5.

X. Gan · J. Janus · V. Dombinov · A. J. Kuhn · D. Reinecke · D. Calahan · L. Nedbal · H. Klose · S. D. Schrey (✉)
Institute of Bio- and Geosciences/Plant Sciences (IBG- 2), Forschungszentrum Jülich, 52425 Jülich, Germany
e-mail: s.schrey@fz-juelich.de

X. Gan
Faculty of Agricultural, Nutritional and Engineering Sciences, University of Bonn, 53115 Bonn, Germany

S. Willbold
Institute of Biological Information Processing (IBI- 7), Forschungszentrum Jülich, 52425 Jülich, Germany

W. Amelung
Institute of Bio- and Geosciences/Agrosphere (IBG- 3), Forschungszentrum Jülich, 52425 Jülich, Germany

W. Amelung
Institute of Crop Science and Resources Conservation, University of Bonn, 53115 Bonn, Germany

D. Calahan
Fycor LLC, Baltimore, MD, USA

H. Klose
RWTH Aachen University, Templergraben 55, 52062 Aachen, Germany

growth-promoting fungus in its interaction with AMF shows the potential and relevance of microbial involvement in using algae-based fertilizers.

Keywords Algal fertilizers · Phosphorus · Wheat · Arbuscular mycorrhizal fungi · *Serendipita vermifera*

Abbreviations

PAB	Polyculture algal biofilms
AMF	Arbuscular mycorrhizal fungi
TSP	Triple superphosphate
ATS	Algal Turf Scrubber
P	Phosphorus
NMR	Nuclear magnetic resonance

Introduction

One of the most urgent problems in agriculture is the supply of nutrients necessary for plant productivity. Phosphorus (P), one of the most essential nutrients for plant growth, is extracted from mined rock phosphate. Since P has an extremely long geological cycle—estimates range from hundreds of millions to billions of years (Smil 2000)—and is thus considered a non-renewable resource, strategies to reduce P losses and recover P from waste streams are crucial to prevent possible shortages in the future. Phosphorus from mineral fertilizers such as ammonium phosphate or superphosphate is highly available to plants, but it can be rapidly immobilized by microbes and fixed as oxides, ultimately limiting its availability to plants (Roy et al. 2016). Additionally, P can be lost through runoff, with eutrophication of water bodies being a severe consequence.

Algae-based bioremediation technologies have emerged as a nature-based solution with considerable nutrient recovery potential (Cai et al. 2013; Calahan et al. 2018). Attached algal biofilm reactors are low-cost devices with a net-like matrix or roughened surface that supports the attachment and growth of polyculture biofilms. Once sufficiently developed, the biofilm can be easily harvested by scraping the accumulated biomass from the matrix. One of the algae biofilm systems, the Algal Turf Scrubber (ATS), has successfully been used for treating liquid manure, agricultural wastewater, and municipal wastewater (Mulbry et al. 2008; Mohsenpour et al. 2021; Ray et al. 2015). An industrial-scale ATS system of 1 ha can produce up to 18 tons of dry biomass per year, recovering up to 0.26 tons of P and 1 ton of nitrogen (N), respectively (Gan et al. 2022, 2023). As the requirements for P recovery from wastewater in Europe become increasingly stringent (Vivienne 2023), algae-based systems could be a valuable option to address this challenge (Hukari et al. 2016).

The polyculture algal biofilm can be an intermediate nutrient carrier between nutrient-rich waters and plant production. Several studies have shown the positive effects of algal fertilizer on plant growth due to its P availability and, to a lesser extent, its N availability (Coppens et al. 2016; Schreiber et al. 2018; Mau et al. 2021, 2022). Yet nutrients must be released from the algal biomass and mineralized to become available to plants. In the soil, decomposers, among them arthropods, protozoa, fungi, and bacteria can break down recalcitrant organic matter and thus initiate the mineralization process (Ekschmitt et al. 2005). Coupled with plant or microbial enzymatic activity, organic P (P_o) is finally converted to inorganic P (P_i) that can be taken up from the plant rhizosphere (Richardson et al. 2011).

Recent studies have investigated the mutual effects of microbial communities and algal biomass on nutrient mobilization in soil (Suleiman et al. 2020; Alobwede et al. 2022) but the interaction of algal biomass with soil fungi has received little attention. This is surprising, as arbuscular mycorrhizal fungi (AMF) in particular can contribute to P nutrition in mycorrhizal plants (Etesami et al. 2021; Martin and van Der Heijden 2024). AMF are predominantly beneficial for P_i uptake since the fungi lack the enzymes required to break down organic biomass, and they exert their effects by exploring soil volumes beyond the ambit of the plant's roots (Hayman and Mosse 1972; Smith and Read 2010). In the presence of P_o, AMF have been shown to cooperate with phosphate-solubilizing bacteria (Etesami et al. 2021; Zhang et al. 2014). While the benefits of organic fertilization over mineral fertilization in terms of soil biological and biochemical properties are well-established (Xie et al. 2014), the effects of organic fertilizers on AMF are less clear, and there is evidence of both beneficial and detrimental effects (Gosling et al. 2006).

The fungal order Sebacinales comprises plant-beneficial root endophytic fungi with high potential for use in agriculture (Ray and Craven 2016; Weiß et al 2016; Varma et al. 2012). Root colonization by members of this order often results in increased host biomass and resistance to biotic and abiotic stresses (Ghimire and Craven 2011; Gill et al. 2016). Sebacinales apparently lack host specificity and have been observed colonizing roots of numerous plant species, including the model organisms *Arabidopsis thaliana*, switchgrass (*Panicum virgatum*), barley, maize, and wheat (Oberwinkler et al. 2013; Ray and Craven 2016). Switching from conventional agriculture to organic management of agricultural fields resulted in a substantial increase in the abundance of members of Sebacinales in wheat roots (Verbruggen et al. 2014). The authors hypothesized that Sebacinales may be sensitive to mineral fertilizers and pesticides or particularly responsive to organic fertilizers. Clearly, the Sebacinales that form mycorrhiza with plants in the Ericaceae family (Selosse et al. 2007) seem to thrive in soils that are rich in organic matter (Verbruggen et al. 2014). The genome of *Serendipita vermifera* contains a range of sequences encoding carbohydrate-active enzymes (CAZymes), indicating that *S. vermifera* has saprotrophic capabilities (Ray et al. 2019). Even though it is still unclear whether CAZymes are expressed during plant-fungus interaction and, if so, whether mobilized nutrients from organic sources are transferred to the host plant, the potential of Sebacinales to contribute to the recovery of organically bound nutrients is intriguing (Ray and Craven 2016; Weiß et al. 2016).

Our first objective was, therefore, to analyze the P species in polyculture algal biofilms (PAB) produced by an ATS system and release of P into the plant growth substrate. To do so, we conducted ^{31}P NMR analyses of PAB, assessed P release via leachate analyses, and carried out a greenhouse experiment to monitor spring wheat (*Triticum aestivum* L., cv. Scirocco) growth and P uptake from PAB. Secondly, we tested the impact of single and dual inoculation of wheat with AMF and *S. vermifera* on plant growth and P uptake from PAB. For this purpose, we conducted a greenhouse experiment with spring wheat fertilized with PAB and mineral fertilizer, inoculated with AMF either alone or in combination with *S. vermifera*. We observed plant growth, P uptake, and root colonization by the fungi.

Materials and methods

Fertilizer

The PAB used as fertilizer in the greenhouse experiments was produced in an ATS system located within a wastewater treatment plant (WWTP; N 51º36′15’’, E 8º46′01’’). The ATS was fed with secondary clarified effluent under local field conditions, as described in Reinecke et al. (2023). The biomass from the ATS was harvested on Aug. 26 th and Sep. 9th, 2021, and pooled. The elemental composition of the PAB was C: 26.54%, N: 3.94%, P: 1.35%, and K: 0.85%. Additional fertilizers for the greenhouse trials were Triplesuperphosphate (TSP, Trifetro Fertilizers, Netherlands), Hakaphos® Gelb, N:P:K 20:0:16, (COMPO EXPERT GmbH, Germany) and potassium sulfate (K_2SO_4, Sigma-Aldrich, USA). Exclusively for the leaching experiment (see below), a second algal biomass with a particularly high P content (nutrient composition C: 17.2%, N: 1.94%, P: 8.70%, and K: 0.28%) was used which was harvested from an ATS situated within a WWTP at Forschungszentrum Jülich (coordinates N 50º54′10’’, E 6º24′14’’). This ATS received effluent directly from the WWTP and operated under field conditions (unpublished).

^{31}P NMR analysis of algal biofilm

PAB-P was extracted by agitating approximately 1 g of dried and milling PAB in a 30 mL extraction solution of 0.25 M NaOH and 50 mM Na_2EDTA for 16 h. The mixture was centrifuged at 14,000 g for 30 min before collecting the supernatant. The supernatant was frozen and lyophilized at − 80 °C. 100 mg of freeze-dried solids was dissolved in 500 µL of a mixture of NaOD and D_2O at pH 13, and 100 µL of methylenediphosphonic acid (MDPA, 0.84 mg mL^{-1} in D_2O) was added as an internal reference. After centrifuging the extracts for 30 min at 14,000 g, solution 1D ^{31}P NMR data were acquired on a Varian 400 MHz spectrometer with a 5 mm broadband probe tuned to the ^{31}P nucleus. Spectra were acquired with the following parameters: 45° pulse calibrated at 9.57 µs, 1.0 s acquisition time, 5 s relaxation delay, 32,678 scans, and proton inverse-gated decoupling at room temperature. Identification of the P species was based on previous measurements of model compounds and natural soils spiked in NaOH-EDTA soil extracts (Missong et al. 2016). The area of each peak was determined to calculate the ratio of P species.

Growth substrate

To represent nutrient-poor substrate with negligible plant-available N, P, and K, a mixture of sand (RBS GmbH, Germany; particle size < 1 mm) and a nutrient-deficient peat-based substrate called "Null Erde" (Einheitserdewerke Werkverband e.V., Germany) was used. The physical properties and nutritional composition of sand and Null Erde (Table S1) are described in detail in Dombinov et al. (2022).

Phosphorus release dynamics from ATS biofilms

The P release dynamics from the fertilizers were assessed following incorporation into the growth substrate (see Section"Growth substrate"). Water and 50 mM citric acid served as extractants. The fertilizers were two different algal biofilms, one that was used in the plant growth experiments containing 1.35% P, and a second containing 8.7% P as well as TSP. Nutrient addition was identical to pot Experiment 2 (see Sections "Response of wheat to algal biofilm fertilization and fungal inoculation (Experiment 2)" and"Growth substrate"). Fifty mL reaction tubes (Falcon, USA) were perforated with several 2 mm diameter holes and filled with 50 g of the substrate and the respective fertilizer blend (plant-free). To adjust water holding capacity (WHC) to 70%, 30 mL of distilled water or 50 mM citric acid were added, respectively. Subsequently, 6–10 mL of distilled water or 50 mM citric acid were added every other day to trigger nutrient leaching. The leachates were collected approximately two hours later, and the volumes (in mL) were recorded. The leachates were analyzed spectrophotometrically (880 nm, SPECORD 200 PLUS, Jena Analytics, Germany) for bioavailable phosphorus (PO_4^{3-}) according to the protocol for ammonium molybdate spectroscopy (ISO, 2004). Leaching with water was carried out for 42 days, whereas leaching with citric acid was stopped after 14 days since almost the entire P content had been released by then. Five replicates were taken of each sample.

Plant growth experiments

Two pot experiments were conducted under controlled conditions in the greenhouse at the Institute for Plant Sciences (IBG- 2), Forschungszentrum Jülich, Germany (N 50°54′36'', E 6 °24′49'') to investigate the effect of PAB and/or fungal inoculation on wheat (*Triticum aestivum*, var. Scirocco, KWS SAAT SE, Germany) growth. The nutrient-poor substrate, i.e., sand/Null Erde mixture, was homogeneously fertilized with PAB or TSP, equivalent to 37 mg P kg^{-1}. As the N and K in PAB were insufficient for wheat growth, Hakaphos® Gelb (containing only N and K) and K_2SO_4 were added at 377.6 mg N kg^{-1} and 303.8 mg K kg^{-1}. Negative control in Experiment 1 was the pure nutrient-poor substrate, while in Experiment 2, the nutrient-poor substrate was supplemented with identical N and K concentrations as the mineral treatment.

The BayEOS sensor (BayCEER, Bayreuth, Germany) measured the light intensity on the plant surface between 6 a.m. and 10 p.m. and the air temperature inside the greenhouse. An automatic drip irrigation system was used to water approximately 60% of the substrate's water-holding capacity with deionized water. All the pots were regularly arranged randomly to ensure a randomized placement of experimental units.

Response of wheat growth to fertilization with algal biofilms (Experiment 1)

Experiment 1 was conducted from April to June 2022 and aimed to investigate the impact of PAB on wheat growth and P uptake. To do so, 18 pots (Ø 17 cm, 2 L) were filled with 1.8 kg of the nutrient-poor substrate (sand:Null Erde, 1:2 *v:v*) containing the fertilization treatments described above. Five wheat seeds germinated in the fertilized substrates were thinned to 3 seedlings per pot after 3 days (d). The plants were grown 9 weeks, and 6 pots per treatment containing 3 plants each were harvested 3, 6, and 9 weeks after seed germination. The developmental stages of the plants were recorded weekly according to the phenological developmental stages of wheat (Meier 2018). All three harvests included dry weight analyses (shoot, root, root mass fractions), and P contents in the biomass were analyzed following the 9-week harvest. The average daily air temperature and light integral were 22.9 °C and 10 mol photons m^{-2} d^{-1}, respectively.

Response of wheat to algal biofilm fertilization and fungal inoculation (Experiment 2)

Experiment 2 was conducted from September to December 2022 and aimed to investigate synergistic or antagonistic effects of PAB and fungal inoculation with *S. vermifera* on wheat growth and P uptake. Three pots (Ø 12 cm, 1 L) per treatment were filled with 0.8 kg of substrate (sand:Null Erde, 1:1 *v:v*) fertilized as described above. Wheat seedlings were pre-germinated for 3 d and inoculated with AMF and *S. vermifera,* individually or in combination (as described below), or remained without inoculation as the negative control. Five pre-germinated seedlings (inoculated depending on the treatment) were planted per pot and thinned to three plantlets after 3 further days. Each treatment comprised 3 replicates.

The inoculation of wheat seedlings was performed as follows: a vermiculite-bound arbuscular mycorrhizal (AMF) inoculum, namely INOQ AGRI, including *Rhizoglomus irregulare, Funneliformis mosseae,* and *Funneliformis geosporum* was obtained from INOQ GmbH (Schnega, Germany) and used according to the manufacturer's instructions. Briefly, 20 mL of inoculum was mixed with the nutrient poor substrate and used as a 5 cm thick top layer into which the seedlings were transplanted. Control treatments received the same amount of vermiculite (Isola Vermiculite, Sprockhövel, Germany) mixed into the substrate. *Serendipita vermifera* (strain MAFF 305830, Syn. *Sebacina vermifera*) was kindly provided by Prof. Alga Zuccaro, Institute for Plant Sciences, University of Cologne, Germany. *S. vermifera* was maintained on MYP medium (7 g L^{-1} of malt extract, 1 g L^{-1} peptone, and 0.5 g L^{-1} yeast extract; Merck, Germany) containing 1.5% agar at 28 °C for 3 weeks as described in (Sarkar et al. 2019). To produce the inoculum for wheat roots, the surface mycelia were scraped off the agar surface with a scalpel and transferred into 100 mL liquid MYP medium and incubated at 23 °C and 120 rpm for 12 d. The mycelium was then carefully crushed with a blender (ULTRA-TURRAX®) and regenerated for a further 3 d. Prior to root inoculation, the hyphal aggregates were poured into a square Petri dish and washed 3 times with sterile tap water. Subsequently, the emerging roots of 3 d old wheat seedlings were gently moved through the solution for 2–3 min to allow fungal material to adhere. The seedlings were immediately carefully planted into the fertilized substrate. All the procedures up to the root dipping were conducted under sterile conditions. The wheat plants were harvested after 6 weeks of growth. The dry weight of roots and shoots and the P content of the biomass were determined, and fungal colonization of wheat roots was evaluated (see below). Throughout the experiment, the average daily air temperature and light integral were 20.4 °C and 5 mol photons m^{-2} d^{-1}, respectively.

Plant harvest and chemical analysis of plant material

The harvested biomass was separated into shoots and roots. Roots were carefully washed to remove the adhering substrate, and both roots and shoots were dried to constant weight at 60 °C (TR 1050, Nabertherm GmbH, Lilienthal, Germany) before measuring dry weights. For Experiment 2, about 0.5 g of the freshly washed roots were sampled from each pot and stored in 15 ml 50% ethanol/water before microscopic assessment of fungal colonization.

In Experiment 1, dry shoots and roots were ground (MM 400, Retsch GmbH, Haan, Germany) collectively to analyze the biomass for P content. In Experiment 2, dry roots and shoots were ground separately to determine if fungal inoculation influenced the distribution of P between the plant organs. Ground biomass was digested with 5 mL HNO_3 in a microwave (MARS6, CEM®, USA) and measured for P by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES Ultima 2, HORIBA®, France).

Quantification of fungal colonization of wheat roots

A non-vital staining procedure was used for the evaluation of mycorrhizal colonization based on Vierheilig et al. (1998). Briefly, root samples were collected at harvest and preserved in 50% ethanol/water. Before staining, roots were washed twice in 50 ml tap water (approx. 15 °C) to remove the ethanol, then cleared by boiling in 10 ml of a 10% potassium hydroxide (KOH) solution for 5 min and rinsed several times with tap water. The samples were boiled for another 3 min in 10 ml of a 5% ink vinegar containing pure white household vinegar (Surig, Mainz, Germany, diluted to 5% acetic acid) and blue Pelikan 4001 ink (Pelikan, Hannover, Germany). The roots were again rinsed with tap water and stored at 4 °C in water with a pH of 4.5 before microscopic observation. Root colonization by AMF was quantified by counting arbuscules, vesicles, hyphae, or fungus-free structures under a light microscope (Zeiss, Germany) as described in McGonigle et al. (1990). In the case of *S. vermifera* inoculation, we identified hyphae and hyphal branches between root cells and root cortical cells completely packed with fungal hyphae, as described for wheat by Ray and Craven (2016). In roots inoculated with both AMF and *S. vermifera*, no effort was made to discriminate the fungal structures, as the hyphae did not show any easily recognizable distinguishable characteristics.

Statistical analysis

R studio, version 2023.06.1 (2023), was used for statistical analyses and generation of figures. Before statistical analysis, the Levene test (R package "heplots", Friendly et al. 2025) was used to test the normality of the data. One-way analysis of variance (ANOVA) was conducted to test the differences among treatments and the statistical significance in the interaction of fertilizers and mycorrhizal applications, followed by Tukey's multiple comparisons test (R package "agricolae", Mendiburu 2023). Differences were considered significant at $p < 0.05$. For variables without a significant interaction between fertilizer and fungal treatments (Figs. 3, 4 and 5A, C, D, E; Fig. S1), separate one-way ANOVAs were conducted for each factor, followed by Tukey's test. In these cases, significant differences between fertilizer treatments are indicated by capital letters, and differences between fungal treatments are indicated by lowercase letters. For variables with a significant interaction (Fig. 5B and F), two-way ANOVA was followed by Tukey's HSD test across all treatment combinations. All data are presented as mean ± standard error (SE). Figures were generated using the R packages ggplot2 (Wickham 2016) and ggpattern (Mike and Davis 2025).

Results

Phosphorus species in PAB

To identify the P species contained in PAB, ^{31}P NMR analyses were conducted. More than 80% of the P extracted by NaOH-EDTA was orthophosphate, and around 15% was present as orthophosphate monoesters. Further species like orthophosphate diesters and pyrophosphate were found in negligible amounts (Fig. 1A). Neither phosphonates nor polyphosphates could be detected. The 1D spectrum is shown in Fig. 1B.

A

Phosphorus compounds	proportion (%)
Orthophosphate	82.9
Orthophosphate-Monoesters	14.7
Orthophosphate-Diesters	0.7
Pyrophosphate	1.8

B

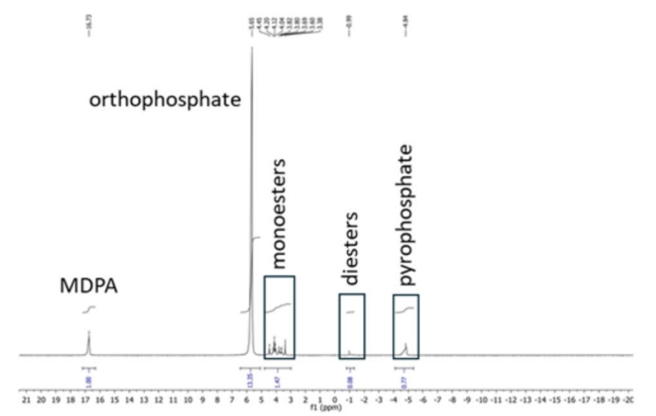

Fig. 1 **A** Percentages of the signal areas and **B**: 1-D spectrum of the P compound classes measured with ^{31}P NMR spectroscopy after EDTA-NaOH extraction

Phosphorus release dynamics

Analyses of leachates collected over 42 days using water as an extractant and of 14 days using 50 mM citric acid revealed differences in P release dynamics between the extractants and the fertilizers (Fig. 2).

PAB linearly released only 1.1% of the total supplied P via water extraction. Hence, mere leaching of P from the PAB is insufficient to feed plants. In contrast, TSP released around 20% of the supplied P over the same time course, with the highest release (15%) occurring before day 14, after which P release gradually decreased. We also tested a PAB containing 8.7% P in addition to the PAB with 1.35% P used for the pot experiments to identify P release dynamics between two contrary PABs. P release of the 8.7% P-containing PAB was also linear over 42 days, providing 3.4% of the P supplied (Fig. 2A). The experiment was repeated for a time course of 14 days using 50 mM citric acid as a root exudate proxy. P release dynamics changed compared to water as an extractant. Using citric acid, the largest amounts of P (2.5%,

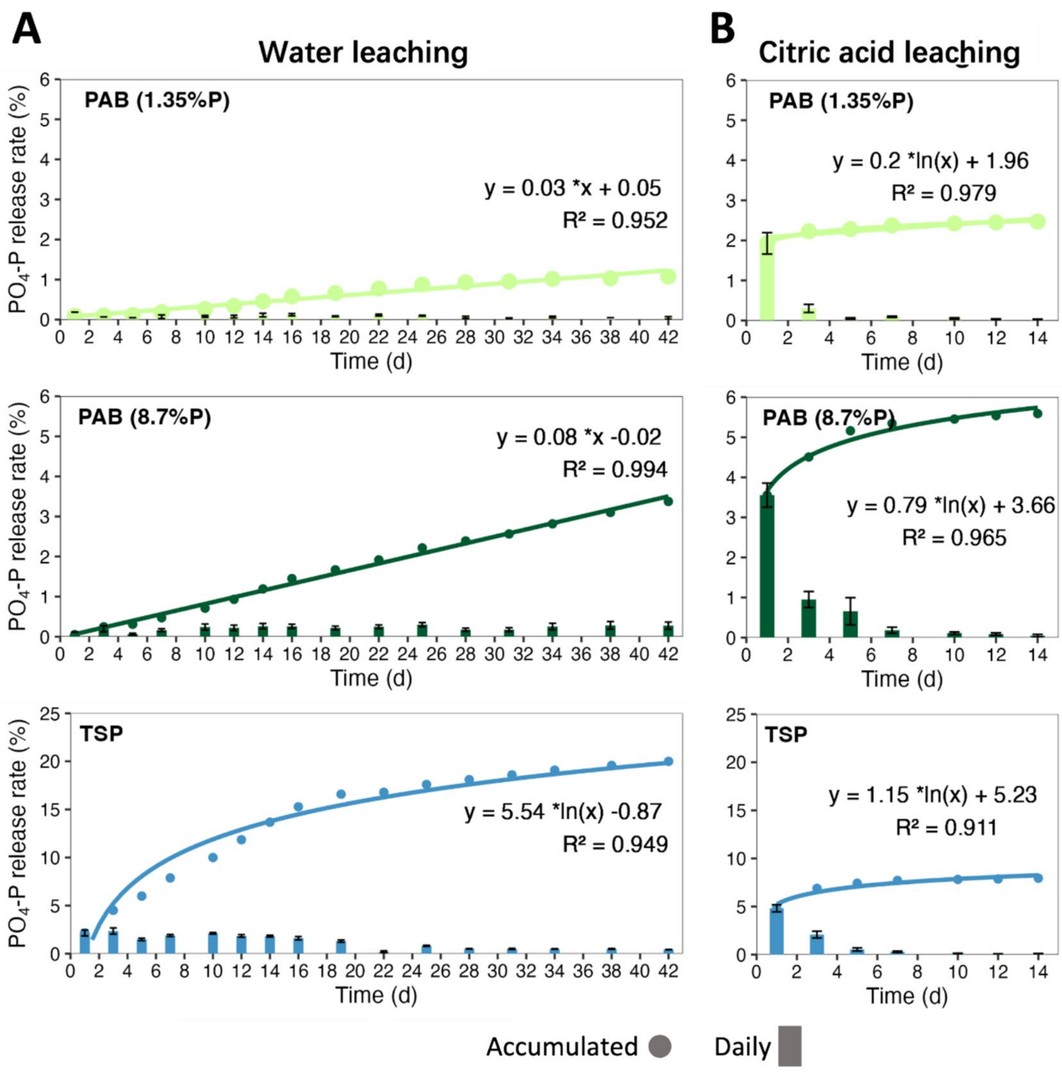

Fig. 2 P mobilization dynamics shown as proportion of total P supplied from a plant-free mixture of growth substrate with PAB (containing 1.35% P and 8.7% P, equivalent to 37 mg kg^{-1} as 100%, light green and dark green, respectively), or TSP (37 mg P kg^{-1} as 100%), leached by water (**A**), 42 days, 6–10 mL d^{-1} or 50 mM citric acid (**B**), 14 days, 6–10 mL d^{-1}. Each graph shows the P release (bars) and the cumulative P release (circles). (n = 5 biological replicates)

5.6% for low and high P PAB, respectively, and 8.0% for TSP) were released within the first five days, after which release decreased, irrespective of the fertilizer (Fig. 2B).

Similar growth performance of PAB- and TSP-fertilized wheat plants despite lower P uptake from PAB (Experiment 1)

This experiment aimed to determine the effect of PAB as a fertilizer for wheat. Wheat phenological development (based on the BBCH scale), dry biomass, and P content (mg P g^{-1} DW) show that fertilized plants performed notably differently from unfertilized plants (Fig. 3). As expected, unfertilized wheat showed a different developmental dynamic (Fig. 3A) and accumulated significantly less biomass than either PAB or TSP fertilized plants at all harvest times (Fig. 3B). Wheat that received P from TSP had slightly but significantly more biomass than PAB fertilized plants at all harvests (Fig. 3B). At the final harvest, the total P content (mg pot^{-1}) of PAB fertilized plants was significantly less than of TSP fertilized plants (Fig. 3C). Relative P (mg g^{-1} DW) of PAB fertilized plants notably but not significantly differed from either negative control or TSP fertilization (Fig. 3D).

Fungal colonization rates differ between fertilizers and fungal inoculum (Experiment 2)

The roots of wheat plants without fungal inoculation showed no fungal structures after 6 weeks of growth (Fig. 4A). In contrast, all plants inoculated either with AMF, *S. vermifera*, or both, showed the typical structures of fungal colonization in the root cortex cells (Fig. 4B-F). Inoculation with AMF resulted in the development of hyphae (Fig. 4B), arbuscules and vesicles (Fig. 4C). In roots inoculated with *S. vermifera*, hyphae and hyphae-filled root cortical cells were counted (Fig. 4D) while chlamydospores were observed but not counted (Fig. 4E). In co-inoculated roots, all structures indicating fungal colonization

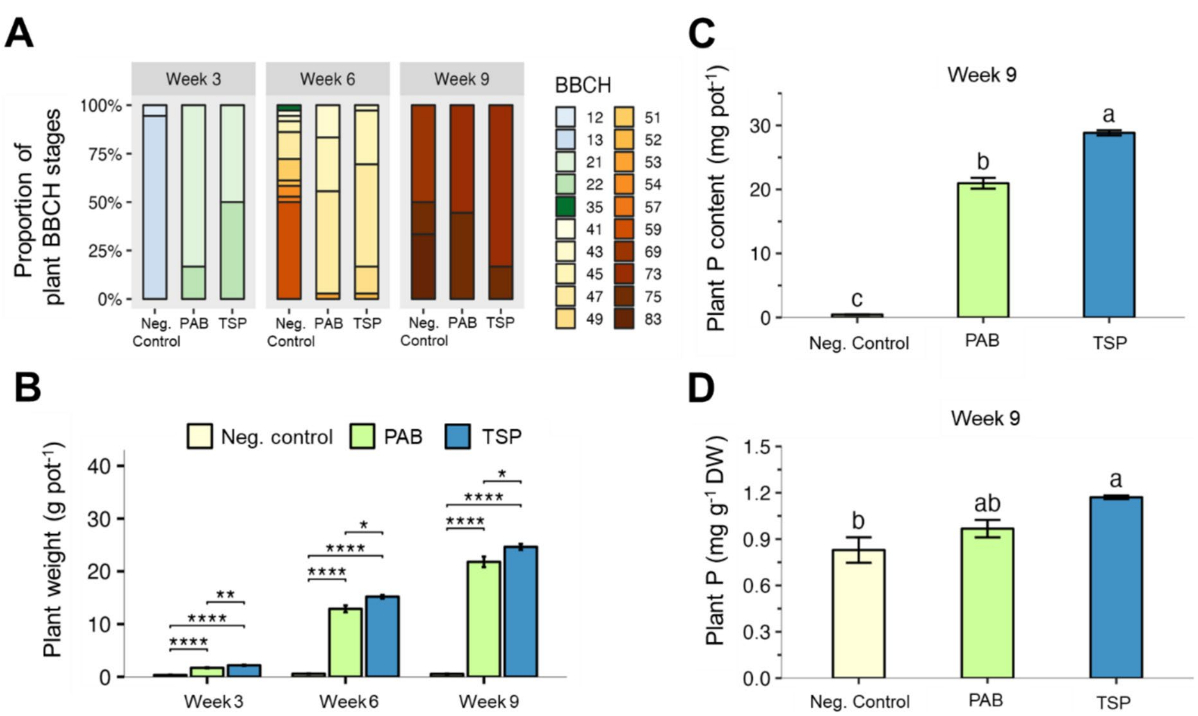

Fig. 3 Development of wheat plants according to BBCH phenological monitoring (**A**), total dry weight of unfertilized (yellow) and algal biofilm (PAB) fertilized (green) or TSP fertilized (blue) wheat plants, harvested after 3, 6 and 9 weeks (**B**), $n = 6$, total P recovered by control, PAB- and TSP-fertilized wheat after 9 weeks (**C**), $n = 3$, and relative P content of wheat plants after harvest at week 9 (**D**), $n = 3$. (Tukey's HSD test, different letters denote significant differences between treatments at $p < 0.05$)

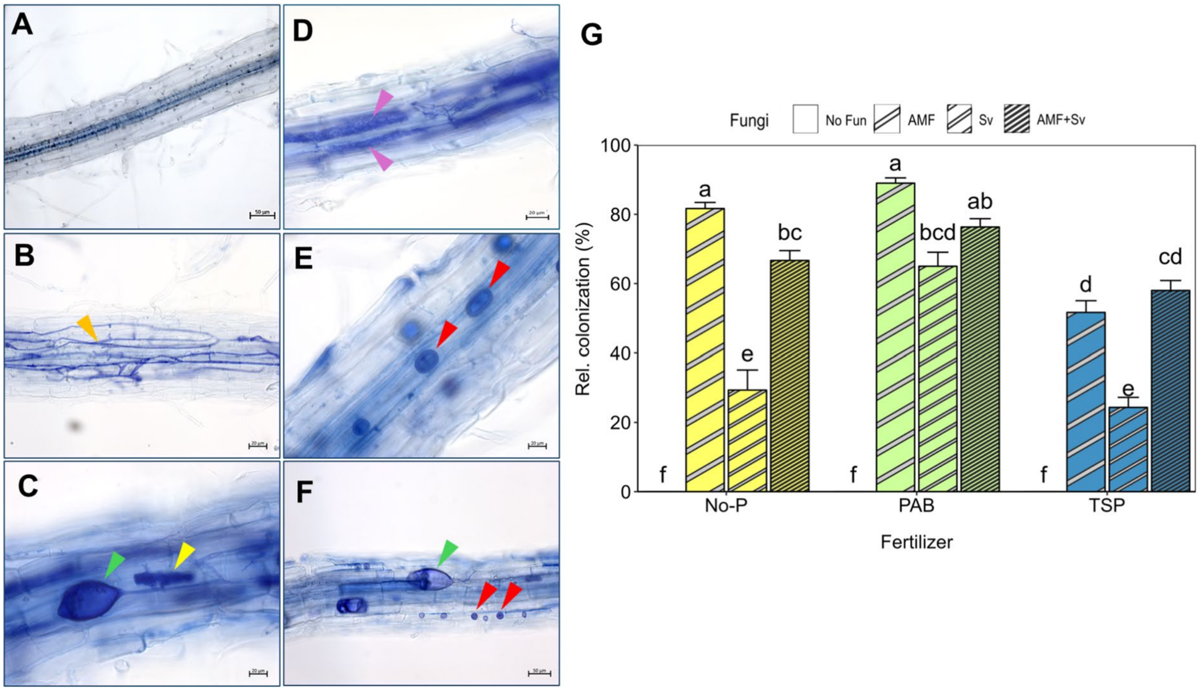

Fig. 4 Exemplary pictures of a typical wheat root segment after staining. **A** no inoculation. Following arbuscular mycorrhizal fungi (AMF) inoculation, the orange arrow in (**B**) points to intracellular hyphae. **C** arbuscules (yellow arrow) and vesicles (green arrow) were typically observed in AMF inoculated roots. In *S. vermifera* (Sv) colonized roots (**D**) hyphae-filled root cells (pink arrows) as well as chlamydospores (**E**, red arrows) were observed. **F** Dual AMF + Sv inoculation showing vesicles (green arrow) and chlamydospores (red arrows). Fungal colonization rate of wheat roots as determined by microscopic observation (**E**). (Tukey's HSD test, different letters denote significant differences between treatments at $p < 0.05$, $n = 3$)

were counted without differentiating between inoculants (Fig. 4F). In all AMF inoculated plants, colonization rates were higher than in *S. vermifera* inoculation alone. In mineral-fertilized plants, the overall colonization rate was lowest, while it was highest in PAB-fertilized plants. Interestingly, the colonization rate with *S. vermifera* was significantly higher in PAB-fertilized plants compared to either TSP- or non-fertilized control (Fig. 4G).

Fungal inoculation differentially affects wheat growth and P uptake from PAB (Experiment 2)

In the second greenhouse experiment, we focused on the effects of fungal inoculation with arbuscular mycorrhizal fungi (AMF), *S. vermifera* (Sv), or both in combination (AMF + Sv), on wheat growth and P uptake from PAB. No-P treatment and TSP fertilization with identical fungal treatments were negative and positive controls, respectively. After 6 weeks of growth, plants were harvested and separated into shoots and roots, followed by measuring growth parameters and P allocation. Significant effects of the treatments are shown in Table 1.

Overall, total dry weight (DW, g pot- 1) and relative P content (mg g- 1 DW) increased depending on the fertilizer treatment (no-*P* < PAB < TSP), with only minor effects of fungal inoculation (Fig. S1). A different pattern emerges when the shoot and root tissues are considered separately (Table 1, Fig. 5). First, both responded differently to fertilizer and fungal treatments: shoot and root parameters (DW, relative and total P content) significantly depended on fertilization ($p < 0.01$ for all parameters), whereas root parameters also showed a fungal inoculation-specific pattern. Second, No-P and PAB fertilization generated a similar pattern in all observed parameters. Third, in TSP fertilization only minor contributions of fungal inoculation to biomass and P content were detected.

Table 1 Results of two-way ANOVA test

Dependent variables	Fungi		Fertilizer		Fungi × Fertilizer	
	F value	*p*	F value	*p*	F value	*p*
Shoot DW (g pot^{-1})	4.409	**0.013**	72.561	**< 0.001**	0.953	0.477
Root DW (g pot^{-1})	49.402	**< 0.001**	60.287	**< 0.001**	9.412	**< 0.001**
P in shoots (mg g^{-1} SDW)	2.754	0.065	23.595	**< 0.001**	0.641	0.696
P in roots (mg g^{-1} RDW)	7.051	**0.001**	6.392	**0.006**	2.232	0.075
Total P in shoots (mg pot^{-1})	1.052	0.388	65.452	**< 0.001**	0.757	0.61
Total P in roots (mg pot^{-1})	20.007	**< 0.001**	31.877	**< 0.001**	6.931	**< 0.001**
DW total plant (g)	7.958	**< 0.001**	77.414	**< 0.001**	1.890	0.124
P in total plant (mg g^{-1} DW)	3.237	**0.04**	24.178	**< 0.001**	0.512	0.793

P-values (*p*) of significant effects ($p < 0.05$) of fungal and/or fertilizer and interaction between them are highlighted in bold

Specifically, the AMF inoculation alone reduced shoot and root DW in No-P control and in PAB fertilized wheat (not significant, Fig. 5A, B). Root DW was significantly promoted by *S. vermifera* in PAB treatment as well as by double inoculation of AMF and *S. vermifera* in No-P and PAB treatment (Fig. 5B). AMF inoculation tended to result in higher relative P content in shoots and roots compared to no AMF inoculation across all fertilizer treatments. In roots, this was significant following PAB fertilization (Fig. 5C, D). In shoots, total P content was highest in TSP fertilized wheat, with no significant effect of fungal inoculation (Fig. 5E) while there was a significant increase in total P in the wheat roots following double inoculation with *S. vermifera* and AMF (Fig. 5F).

Regarding the effects of fungal inoculation on wheat performance following PAB fertilization this shows that AMF moderately enhanced P uptake but also negatively affected plant growth. In contrast, *S. vermifera* alone had no effect on P uptake but instead promoted root growth. Double inoculation with both fungi resulted in higher total P uptake from PAB, resulting in significantly more total P content in wheat roots than following TSP fertilization, used here as a positive control.

Discussion

In this study we show the potential and limitations of a polyculture algal biofilm (PAB) produced on the effluent of treated municipal wastewater as a phosphorus (P) fertilizer for wheat. Although orthophosphate is the main P species in the NaOH labile P fraction of PAB, it was less prone to leaching and less available to wheat plants during 42 days of growth compared to TSP. We further show that the co-inoculation of wheat with arbuscular mycorrhizal fungi (AMF) and the root-beneficial fungus *Serendipita vermifera* resulted in a comparable fertilizer efficiency of PAB and TSP, which was due to a synergistic effect of root growth promotion by *S. vermifera* and enhanced P mobilization by AMF.

^{31}P NMR analysis reveals orthophosphate as the dominant P species in PAB

The NaOH-extractable fraction of P from PAB consisted mainly of orthophosphate (83%), followed by orthophosphate-monoesters (15%), pyrophosphate (2%), and orthophosphate-diesters (< 1%; Fig. 1). The same P species but also phosphonates and polyphosphates were previously detected in algae (Wang et al. 2019). In our study, we did not detect phosphonates and polyphosphates, which could have been due to different cultivation conditions, differences in the algae community, and/or their amounts being below the limit of quantification. In addition, polyphosphate may have been hydrolyzed during alkaline extraction, resulting in its conversion to pyrophosphate (He et al. 2011). To test such underlying processes, Wang et al. (2021) had also extracted the algae with a deuterated solution, which allowed quantification of hydrolytic decay based on changes in chemical shifts at the slightly modified electron density of the nuclei. The authors reported that algal P also contained a significant amount of RNA-P (1.260 g kg^{-1}; in similar amounts as monoester-P) in addition to some phospholipid P (340 mg kg^{-1}), which both escape common analytical windows using alkaline extraction.

NaOH-EDTA has been widely used to extract P from environmental samples. In soils and sediments,

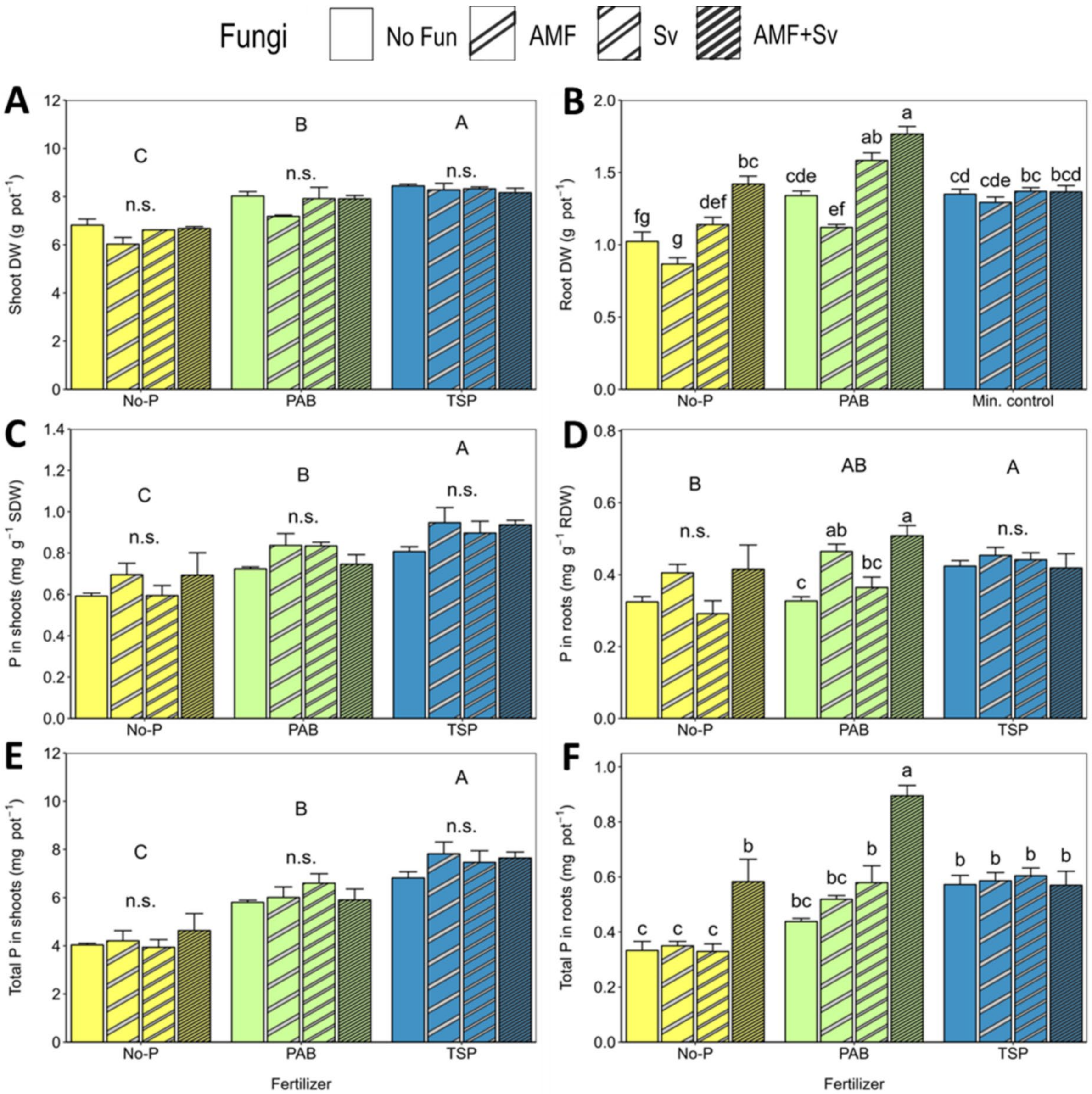

Fig. 5 Effects of fertilizer and fungal treatments on growth and phosphorus uptake of wheat plants. **A** Shoot dry weight, **B** Root dry weight, **C** Shoot P concentration, **D** Root P concentration, **E** Total P in shoots, and (**F**) Total P in roots. For panels with no significant interaction (**A**, **C**, **D**, **E**), significant differences between fertilizer treatments are indicated by capital letters (No-P, BAP, TSP, $p < 0.01$, $n = 12$), and differences between fungal treatments are indicated by lowercase letters (No-Fun, AMF, Sv, AMF + Sv, $p < 0.05$, $n = 3$), both based on one-way ANOVA followed by Tukey's test. For panels with significant interaction (**B**, **F**), different letters indicate significant differences between treatment combinations, based on two-way ANOVA followed by Tukey's test. Error bars represent standard errors (SE)

extraction of P with 0.25 M NaOH and 50 mM EDTA resulted in recovery rates between 57 and 90% of total P (Makarov et al. 2002; Missong et al. 2016). Although the extraction method was optimized for soil samples, it has also been used successfully for algal biomass (Feng et al. 2016, 2019). Feng et al. (2019) applied the extraction solution to aquatic macrophytes and algae and achieved a P recovery of around 90%. Mackay et al. (2017) observed that the high amounts of orthophosphate in different organic soil amendments did not always correspond to higher plant availability. Instead, the composition and changes within the P pools over time had a governing effect on plant available P. Similarly, Peirce et al. (2013) compared P species in chicken manure over a 12-month period and identified increasing concentrations of orthophosphate with increasing age, which corresponded to reduced P availability to plants. The authors suggested increased binding of the orthophosphate to calcium in the substrate. Algal biofilms produced on municipal wastewater may contain around 2% calcium (Reinecke et al. 2023), which may, upon decomposition of algal biomass, immobilize orthophosphate. Furthermore, Schreiber et al. (2018) analyzed P availability from algal biomass composed of *Chlorella vulgaris* and observed differences between dry and wet algae as well as sandy and organic-rich substrates, indicating effects of biomass processing and growing medium on the P availability to plants. Other than orthophosphate, the PAB contains negligible amounts (< 1%) of the easily mineralizable orthophosphate diester-P (Condron et al. 1990) and around 2% of less plant available pyrophosphate (Sutton and Larsen 1964), while around 15% was in the form of orthophosphate monoester. Monoesters may readily sorb to soil surfaces, making them stable, and they are assumed to be less susceptible to mineralization (Giles et al. 2011; Turner et al. 2005). Plants utilize P in the form of inorganic orthophosphate (Pi), and the high orthophosphate concentration of PAB would be expected to correspond to high plant availability (Penn and Camberato 2019). However, as discussed below (4.3), plant P availability was consistently lower than from TSP, which likely reflects that much of the PAB total P was organic and thus not readily taken up.

P release dynamics from PAB with water and citric acid as extractants predict low leaching and high plant availability of PAB-P

The leaching experiment conducted with PAB or TSP mixed into the plant-free growing medium showed differences in the P release dynamics between the fertilizers. PAB-P was much less water soluble than the (monocalcium)-P from TSP. This could reflect the presence of other, less leaching-prone P forms in PAB, such as the 15% orthophosphate monoester share discussed above (4.1). However, the leaching dynamics were not different between a PAB containing 1.35% and one containing 8.7% P; only the absolute amounts differed. This suggests that the P species in both algal biofilms are similar. The high P content in the PAB may result from P precipitation as hydroxyapatite during high-light and high-temperature conditions (such as in summer days) when the pH of the medium rises to values above pH 9 (for details, see Gerardi and Lytle 2015); such high pH values have also been observed in the ATS system by Gan et al. (2023). Alternatively, due to so-called"luxury uptake", algal cells can accumulate much more P than is necessary for their immediate growth and to store it in the form of polyphosphate. The stored P can serve as an internal P depot when needed (Solovchenko et al. 2016). The analyses of the 1.35% P PAB detected no polyphosphate but further studies on the high P-PAB would help to identify the P species and their localization, allowing for the optimization of PAB as a fertilizer.

The slow P release from PAB contrasts with the comparatively high P uptake by the plants (see 4.3), suggesting that other, probably plant-related processes contributed to P acquisition. On the one hand, P may be rapidly released from organic forms upon mineralization, as other studies also showed similar P availability to plants from algae as from TSP (Schreiber et al. 2018; Siebers et al. 2019). On the other hand, to become available to plants, P that is adsorbed to soil or precipitated must be desorbed or solubilized from the pool of total P (Richardson et al. 2011). To improve the access to such P, plants utilize several mechanisms including the exudation of low molecular mass organic acids that not only release phosphate from its binding sites but also dissolve hydroxyapatite P, thus increasing the availability of P to plants (Wang and Lambers 2020). The short-term leaching experiment conducted here with 50 mM citric acid, a commonly exuded organic acid frequently used to study P dynamics in soils (Santos et al. 2017), shows that the release dynamics changed compared to water as an extractant, resembling the dynamics observed in P leaching from TSP. With water, PAB-P was released gradually, while with citric acid, the largest amounts of P were released within the first few days, after which its release decreased, irrespective of the fertilizer (Fig. 1B). Citric acid can be considered a proxy for acidic root exudates, and its effect indicates that PAB-P can become plant-available in the presence of a root.

P from PAB is readily available for wheat, but to a notably smaller extent than P from TSP

Wheat phenological development, assessed via the BBCH scale, indicated comparable development following both fertilizer treatments at weeks 3, 6, and 9, compared to the unfertilized control. Overall, plant biomass and the total and relative P of control plants remained significantly different from that of the fertilized plants at all time points (Fig. 2). Comparing the fertilized plants, wheat receiving PAB yielded less biomass and acquired significantly less total phosphorus compared to wheat fertilized with TSP. The relative P content, while notably lower than that following TSP treatment, was not significant, suggesting comparable P availability to that of TSP.

As discussed above, this aligns with our expectation following the ^{31}P NMR and P-leaching results that indicated that PAB-P could be slightly less available than TSP-P. These results are further in accordance with previous reports on algal fertilizers (Schreiber et al. 2018; Siebers et al. 2019). Mau et al. (2021) used phenological data and literature-derived information to analyse wheat growth with an algal fertilizer composed of *Chlorella* sp. Their findings indicate that a portion of the P was readily accessible from the algal biomass, while another fraction was released gradually over time. This resulted in wheat growth comparable to that of plants fertilized with minerals, albeit with a lower overall P uptake. However, previous studies have exclusively examined the impacts of algal monocultures, while we demonstrate successful plant growth using a polyculture algal biofilm.

Double inoculation of wheat with arbuscular mycorrhizal fungi and the beneficial root endophyte *Serendipita vermifera* improved P transfer from PAB to the roots, which retained more P than from readily available TSP

Our next aim was to analyse whether and to what extent wheat P uptake can benefit from the synergy between arbuscular mycorrhizal fungi (AMF) and *S. vermifera* when PAB is used as fertiliser. The contribution of AMF to plant P uptake is well understood (Jansa et al. 2011; Smith and Read 2010), while it is much less clear for *S. vermifera* and other members of the Sebacinales. P acquisition by host plants can be enhanced under some experimental conditions, but the mechanisms differ from those identified in AMF (Weiß et al. 2016). To gain insight into the interaction of wheat with both types of beneficial fungi, we inoculated wheat seedlings with AMF and *S. vermifera*, alone and in combination. We harvested the roots and shoots after 6 weeks of growth. Independently of the fertilizer treatment, all typical mycorrhizal structures could be detected in AMF-inoculated, as well as in *S. vermifera*-inoculated roots, indicating the establishment of functional root-fungus interactions (Fig. 4).

Using PAB as a fertilizer resulted in significantly higher root colonization with *S. vermifera* compared to TSP fertilization and the No-P treatment. Sebacinales have been found to proliferate in soils that are high in organic matter and were also found to increase following a transition from conventional to organic agriculture (Verbruggen et al. 2014). In our experiment, potential proliferation of *S. vermifera* in the presence of PAB may have led to higher fungal biomass and thus increased inoculum density in the substrate. In AMF (i.e. *Glomus* spp.), root infection levels can be proportional to inoculum amount (Wilson and Trinick 1983), although colonization also depends on the fungus's infection competence (Abbott and Robson 1981). In addition, we observed that *S. vermifera* inoculation increased wheat root biomass (see below), which potentially also increased the number of entry points for fungal colonization. Follow-up experiments would be needed to clarify the roles of inoculum density, infection competence, and root physiological responses.

It is interesting to note that the presence or absence of P did not affect the root colonization rate, as there was no difference in *S. vermifera* colonization between the No-P and TSP fertilization treatments. With double inoculation, *S. vermifera* did not affect AMF colonization in PAB and TSP treatments while significantly lower colonization was observed in the No-P treatment compared to AMF inoculation alone. This suggests that *S. vermifera* may inhibit AMF's ability to colonize wheat roots at extremely low P availability. Sarkar et al. (2019) studied *S. vermifera* in the interaction with the plant pathogenic fungus *Bipolaris sorokiniana*. The authors found that genes encoding cell wall-degrading enzymes, particularly chitinases and glucanases, were upregulated in *S. vermifera* during confrontation with *B. sorokiniana*. Since the identified gene products are important for mycoparasitism (Sarkar et al. 2019), it is feasible to assume that such antagonism also may play a role in the interaction between AMF and *S. vermifera,* which may in turn affect the ability of AMF to colonize plant roots.

AMF inoculation had a negative effect on plant biomass formation, while the relative P content increased (albeit not significantly) compared to non-inoculated plants (Fig. 5). In wheat, which is often regarded as non-responsive to AMF, such an outcome has been described before. However, previous researchers have demonstrated that considerable amounts of P were delivered by AMF to wheat, even when the growth response was negative (Li et al. 2006, 2005). This indicates that the growth response may not be suitable for judging the fungal contribution to P-use efficiency of plants.

In our study, *S. vermifera* alone did not affect P uptake but significantly promoted wheat root growth following PAB fertilization. *S. vermifera* promotes the growth of many different plant hosts, including the model plants *Arabidopsis* and *Nicotiana,* but also the biomass plant switchgrass (*Panicum virgatum*), fennel (*Foeniculum vulgare*), and rice (Dolatabadi et al. 2011; Pirdashti et al. 2012; Ray and Craven 2016). Achatz et al. (2010) reported that growth promotion in barley (*Hordeum vulgare*) by *Serendipita indica* (syn. *Pirformospora indica*), a close relative of *S. vermifera*, was not accompanied by increased P content. Other members of the order Sebacinales in contrast have been shown to provide N and P to host plants, especially when these nutrients were limited (Nurfadilah et al. 2013). Ray et al. (2019) reported a significant increase in the number of lateral roots in young wheat seedlings in winter wheat inoculated with *S. vermifera ssp. bescii* under N- or P-limiting conditions. However, under these conditions no impact on shoot N or P content conditions was observed, and, in turn, no growth promotion under optimum N and P conditions was evident. The authors suggested that shoot nutrient acquisition and plant growth promotion may not necessarily be positively correlated phenotypic traits. In the wheat-*S. vermifera* interaction introduced in our study, similar dynamics could be at play (Fig. 5).

Co-inoculation of AMF and *S. vermifera* led to a significant increase in root P content but only a slightly positive, non-significant trend concerning the aboveground biomass. Positive synergistic effects of co-inoculation of plants with AMF and *S. indica* have been described that alleviate saline stress (Heidarianpour et al. 2020), drought stress (Tyagi et al. 2023), and to reduce heavy metal uptake (Wang et al. 2023). He et al. (2022) co-inoculated the legume *Vicia villosa* with AMF and *S. indica,* resulting in increased plant biomass, a significantly improved P uptake and increased nitrogen (N) availability in the soil. Similarly, Hallasgo et al. (2020) studied the synergistic effects of AMF and two different endophytes, *S. indica* and *S. williamsii*, on tomato plants. Double inoculation enhanced the N concentration in tomato shoots without reducing the P transfer by AMF. Moreover, the authors observed that *S. williamsii* performed differently from *P. indica* when co-inoculated with AMF, indicating that interaction patterns, even between close relatives, are highly individual. Here, we used an AMF inoculum consisting of three arbuscular mycorrhizal fungi, namely *Rhizoglomus irregulare, Funneliformis mosseae,* and *Funneliformis geosporum,* and we cannot exclude that the individual AMF species interact differently with *S. vermifera* and that individual effects may be lost in the combination. Thus, it would be informative to further investigate specific interactions with *S. vermifera* as, to our knowledge, no other studies on potential synergism or antagonism between *S. vermifera* and AMF isolates have been conducted.

In summary we show that P recovery from PAB by wheat plants can be enhanced by co-inoculation with arbuscular mycorrhizal fungi and the beneficial root endophytic fungi *S. vermifera*. Even though under the chosen experimental conditions there was no positive effect on overall plant biomass, more total P was retained in belowground organic plant matter, preventing it from being immobilized in the soil. Li et al. (2005) reported that the wheat growth depression induced by AMF observed at early growth stages (up to 6 weeks) disappeared at maturity. It would thus be essential to conduct next experiments until wheat maturity to evaluate the potential of dual inoculation on the wheat seed production, quality, and P recovery from PAB.

Conclusion

In our study, PAB proved to be an effective organic P source for wheat, with lower P-leaching potential than TSP. Despite wheat's lower P recovery from PAB compared to TSP, it grew efficiently with this treatment. While AMF reduced wheat growth without affecting P uptake in PAB-fertilized plants, *S. vermifera* mitigated the adverse effects when co-inoculated with AMF. Notably, *S. vermifera* significantly enhanced root growth and P content in roots. Our study thus highlights the potential of microbial involvement in optimizing the use of algae-based fertilizers for sustainable agriculture. It contributes to the growing understanding of algae-based fertilizers in plant nutrition.

As Sebacinales are more abundant in organically managed soils than in conventionally cultivated ones, PAB might have specific advantages in joint application with organic fertilizers, at best reducing the necessity to inoculate the crops. It should also be noted that mineral fertilization as well as the absence of P inhibited *S. vermifera* colonization of wheat roots, while the presence of *S. vermifera* inhibited AMF colonization under very low P conditions. Fertilizer, soil, or organismic factors involved in this tripartite interaction need further elucidation.

Acknowledgements The authors would like to thank Prof. Dr. Alga Zuccaro, University of Cologne, for the kind donation of a culture of *Serendipita vermifera* strain MAFF305830. XG was supported by the China Scholarship Council [grant number 201906230353].

Author contributions Conceptualization: SDS, AJK, WA; Methodology, investigation, and formal analysis: XG, JJ, SW, VD, SDS; Writing—original draft preparation: SDS; Writing—review and editing: all authors; Funding acquisition: LN; Resources: HK, DLR; Supervision: SDS, DLR, SDC, LN. All authors have read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Abbott LK, Robson AD (1981) Infectivity and effectiveness of vesicular arbuscular mycorrhizal fungi: effect of inoculum type. Aust J Agric Res 32(4):631–639

Achatz B, von Rüden S, Andrade D, Neumann E, Pons-Kühnemann J, Kogel K-H, Franken P, Waller F (2010) Root colonization by Piriformospora indica enhances grain yield in barley under diverse nutrient regimes by accelerating plant development. Plant Soil 333:59–70

Alobwede E, Cotton A, Leake JR, Pandhal J (2022) The fate and distribution of microalgal nitrogen when applied as an agricultural soil fertiliser and its effect on soil microbial communities. Phycology 2(3):297–318

Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew Sustain Energy Rev 19:360–369

Calahan D, Osenbaugh E, Adey W (2018) Expanded algal cultivation can reverse key planetary boundary transgressions. Heliyon 4(2):e00538

Condron L, Frossard E, Tiessen H, Newmans R, Stewart J (1990) Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental conditions. J Soil Sci 41(1):41–50

Coppens J, Grunert O, Van Den Hende S, Vanhoutte I, Boon N, Haesaert G, De Gelder L (2016) The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J Appl Phycol 28:2367–2377

Dolatabadi HK, Goltapeh EM, Jaimand K, Rohani N, Varma A (2011) Effects of Piriformospora indica and Sebacina vermifera on growth and yield of essential oil in fennel (Foeniculum vulgare) under greenhouse conditions. J Basic Microbiol 51(1):33–39

Dombinov V, Herzel H, Müller F, Willbold S, Zang JW, da Fonseca-Zang WA, Klose H, Poorter H, Jablonowski ND, Schrey SD (2022) Sugarcane bagasse ash as fertilizer for soybeans: Effects of added residues on ash composition, mineralogy, phosphorus extractability and plant availability. Front Plant Sci 13:1041924

Ekschmitt K, Liu M, Vetter S, Fox O, Wolters V (2005) Strategies used by soil biota to overcome soil organic matter stability—why is dead organic matter left over in the soil? Geoderma 128(1–2):167–176

Etesami H, Jeong BR, Glick BR (2021) Contribution of arbuscular mycorrhizal fungi, phosphate–solubilizing bacteria, and silicon to P uptake by plant. Front Plant Sci 12:699618

Feng W, Zhu Y, Wu F, Meng W, Giesy JP, He Z, Song L, Fan M (2016) Characterization of phosphorus forms in lake macrophytes and algae by solution 31 P nuclear magnetic resonance spectroscopy. Environ Sci Pollut Res 23:7288–7297

Feng W, Li C, Zhang C, Liu S, Song F, Guo W, He Z, Li T, Chen H (2019) Characterization of phosphorus in algae from a eutrophic lake by solution 31 P nuclear magnetic resonance spectroscopy. Limnology 20:163–171

Friendly M, Fox J, Monette G (2025) heplots: Visualizing Tests in Multivariate Linear Models. R package version 1.7.5. URL. Available at: https://CRAN.R-project.org/package=heplots. Accessed 30 Apr 2025

Gan X, Klose H, Reinecke D (2022) Optimizing nutrient removal and biomass production of Algal Turf Scrubber (ATS) under variable cultivation conditions by using response surface methodology. Front Bioeng Biotechnol 10:1265

Gan X, Klose H, Reinecke D (2023) Stable year-round nutrients removal and recovery from wastewater by technical-scale algal Turf Scrubber (ATS). Separ Purification Technol 307:122693

Gerardi MH, Lytle B (2015) Algae, Alkalinity, and pH. In: The Biology and Troubleshooting of Facultative Lagoons, 105–109. Eds: xxx https://doi.org/10.1002/9781118981771.ch13

Ghimire SR, Craven KD (2011) Enhancement of switchgrass (Panicum virgatum L.) biomass production under drought conditions by the ectomycorrhizal fungus Sebacina vermifera. Appl Environ Microbiol 77(19):7063–7067

Giles C, Cade-Menun B, Hill J (2011) The inositol phosphates in soils and manures: abundance, cycling, and measurement. Can J Soil Sci 91(3):397–416

Gill SS, Gill R, Anjum NA, Sharma KK, Johri AK, Tuteja N (2016) Piriformospora indica: potential and significance in plant stress tolerance. Front Microbiol 7:184779

Gosling P, Hodge A, Goodlass G, Bending G (2006) Arbuscular mycorrhizal fungi and organic farming. Agr Ecosyst Environ 113(1–4):17–35

Hallasgo AM, Spangl B, Steinkellner S, Hage-Ahmed K (2020) The fungal endophyte Serendipita williamsii does not affect phosphorus status but carbon and nitrogen dynamics in arbuscular mycorrhizal tomato plants. Journal of Fungi 6(4):233

Hayman D, Mosse B (1972) Plant growth responses to vesicular-arbuscular mycorrhiza III. Increased uptake of labile p from soil. New Phytol 71(1):41–47

He Z, Olk DC, Cade-Menun BJ (2011) Forms and lability of phosphorus in humic acid fractions of Hord silt loam soil. Soil Sci Soc Am J 75(5):1712–1722

He W-X, Wu Q-S, Hashem A, Abd_Allah EF, Muthuramalingam P, Al-Arjani A-B.F, Zou Y-N (2022) Effects of symbiotic fungi on sugars and soil fertility and structure-mediated changes in plant growth of Vicia villosa. Agriculture 12(10):1523

Heidarianpour MB, Aliasgharzad N, Olsson PA (2020) Positive effects of co-inoculation with Rhizophagus irregularis and Serendipita indica on tomato growth under saline conditions, and their individual colonization estimated by signature lipids. Mycorrhiza 30(4):455–466

Hukari S, Hermann L, Nättorp A (2016) From wastewater to fertilisers—Technical overview and critical review of European legislation governing phosphorus recycling. Sci Total Environ 542:1127–1135

Jansa J, Finlay R, Wallander H, Smith FA, Smith SE (2011) Role of mycorrhizal symbioses in phosphorus cycling. Phosphorus in action: biological processes in soil phosphorus cycling, pp 137–168

Li H, Zhu Y, Marschner P, Smith F, Smith S (2005) Wheat responses to arbuscular mycorrhizal fungi in a highly calcareous soil differ from those of clover, and change with plant development and P supply. Plant Soil 277:221–232

Li H, Smith SE, Holloway RE, Zhu Y, Smith FA (2006) Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. New Phytol 172(3):536–543

Mackay J, Macdonald L, Smernik R, Cavagnaro T (2017) Organic amendments as phosphorus fertilisers: Chemical analyses, biological processes and plant P uptake. Soil Biol Biochem 107:50–59

Makarov M, Haumaier L, Zech W (2002) The nature and origins of diester phosphates in soils: a 31 P-NMR study. Biol Fertil Soils 35:136–146

Martin FM, van Der Heijden MG (2024) The mycorrhizal symbiosis: research frontiers in genomics, ecology, and agricultural application. New Phytol

Mau L, Kant J, Walker R, Kuchendorf CM, Schrey SD, Roessner U, Watt M (2021) Wheat can access phosphorus from algal biomass as quickly and continuously as from mineral fertilizer. Front Plant Sci 12:631314

Mau L, Junker S, Bochmann H, Mihiret YE, Kelm JM, Schrey SD, Roessner U, Schaaf G, Watt M, Kant J (2022) Root growth and architecture of wheat and brachypodium vary in response to algal fertilizer in soil and solution. Agronomy 12(2):285

McGonigle TP, Miller MH, Evans D, Fairchild G, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol 115(3):495–501

Meier U (2018) Entwicklungsstadien mono-und dikotyler Pflanzen: BBCH Monografie (H. Bleiholder, L. Buhr, C. Feller, H. Hack, M. Heß, R. Klose, P. D. Lancashire, U. Meier, R. Stauß, T. van den Boom, & E. Weber). Open Agrar Repositorium. https://doi.org/10.5073/20180906-075119

Mendiburu F (2023) Agricolae: Statistical procedures for agricultural research. Available at: https://cran.r-project.org/web/packages/agricolae/index.html

Mike FC, Davis TL (2025) ggpattern: 'ggplot2' Pattern Geoms. R package version 1.1.4, https://trevorldavis.com/R/ggpattern/, accessed 30 Apr 2025. Available at: https://cran.r-project.org/web/packages/ggpattern/ggpattern.pdf

Missong A, Bol R, Willbold S, Siemens J, Klumpp E (2016) Phosphorus forms in forest soil colloids as revealed by liquid-state 31P-NMR. J Plant Nutr Soil Sci 179(2):159–167

Mohsenpour SF, Hennige S, Willoughby N, Adeloye A, Gutierrez T (2021) Integrating micro-algae into wastewater treatment: A review. Sci Total Environ 752:142168

Mulbry W, Kondrad S, Pizarro C, Kebede-Westhead E (2008) Treatment of dairy manure effluent using freshwater algae: Algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. Biores Technol 99(17):8137–8142

Nurfadilah S, Swarts ND, Dixon KW, Lambers H, Merritt DJ (2013) Variation in nutrient-acquisition patterns by mycorrhizal fungi of rare and common orchids explains diversification in a global biodiversity hotspot. Ann Bot 111(6):1233–1241

Oberwinkler F, Riess K, Bauer R, Selosse M-A, Weiß M, Garnica S, Zuccaro A (2013) Enigmatic sebacinales. Mycol Prog 12:1–27

Peirce C, Smernik R, McBeath T (2013) Phosphorus availability in chicken manure is lower with increased stockpiling period, despite a larger orthophosphate content. Plant Soil 373:359–372

Penn CJ, Camberato JJ (2019) A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. Agriculture 9(6):120

Pirdashti H, Yaghoubian Y, Goltapeh E, Hosseini S (2012) Effect of mycorrhiza-like endophyte (Sebacina vermifera) on growth, yield and nutrition of rice (Oryza sativa L.) under salt stress. J Agric Technol 8(5):1651–1661

Ray P, Craven KD (2016) Sebacina vermifera: a unique root symbiont with vast agronomic potential. World J Microbiol Biotechnol 32:1–10

Ray NE, Terlizzi DE, Kangas PC (2015) Nitrogen and phosphorus removal by the Algal Turf Scrubber at an oyster aquaculture facility. Ecol Eng 78:27–32

Ray P, Abraham PE, Guo Y, Giannone RJ, Engle NL, Yang ZK, Jacobson D, Hettich RL, Tschaplinski TJ, Craven KD (2019) Scavenging organic nitrogen and remodelling lipid metabolism are key survival strategies adopted by the endophytic fungi, Serendipita vermifera and Serendipita bescii to alleviate nitrogen and phosphorous starvation in vitro. Environ Microbiol Rep 11(4):548–557

Reinecke D, Bischoff L-S, Klassen V, Blifernez-Klassen O, Grimm P, Kruse O, Klose H, Schurr U (2023) Nutrient recovery from wastewaters by algal biofilm for fertilizer production part 1: Case study on the techno-economical aspects at pilot-scale. Sep Purif Technol 305:122471

Richardson AE, Lynch JP, Ryan PR, Delhaize E, Smith FA, Smith SE, Harvey PR, Ryan MH, Veneklaas EJ, Lambers H (2011) Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349:121–156

Roy ED, Richards PD, Martinelli LA, Coletta LD, Lins SRM, Vazquez FF, Willig E, Spera SA, VanWey LK, Porder S (2016) The phosphorus cost of agricultural intensification in the tropics. Nat Plants 2(5):1–6

Santos SR, de Barros Silva E, Alleoni LRF, Grazziotti PH (2017) Citric acid influence on soil phosphorus availability. J Plant Nutr 40(15):2138–2145

Sarkar D, Rovenich H, Jeena G, Nizam S, Tissier A, Balcke GU, Mahdi LK, Bonkowski M, Langen G, Zuccaro A (2019) The inconspicuous gatekeeper: endophytic Serendipita vermifera acts as extended plant protection barrier in the rhizosphere. New Phytol 224(2):886–901

Schreiber C, Schiedung H, Harrison L, Briese C, Ackermann B, Kant J, Schrey SD, Hofmann D, Singh D, Ebenhöh O (2018) Evaluating potential of green alga Chlorella vulgaris to accumulate phosphorus and to fertilize nutrient-poor soil substrates for crop plants. J Appl Phycol 30(5):2827–2836

Selosse MA, Setaro S, Glatard F, Richard F, Urcelay C, Weiß M (2007) Sebacinales are common mycorrhizal associates of Ericaceae. New Phytol 174(4):864–878

Siebers N, Hofmann D, Schiedung H, Landsrath A, Ackermann B, Gao L, Mojzeš P, Jablonowski ND, Nedbal L, Amelung W (2019) Towards phosphorus recycling for agriculture by algae: Soil incubation and rhizotron studies using 33P-labeled microalgal biomass. Algal Res 43:101634

Smil V (2000) Phosphorus in the environment: natural flows and human interferences. Ann Rev Energy Environ 25(1):53–88

Smith SE, Read DJ (2010) Mycorrhizal symbiosis, 3rd edn. Academic press, New York, USA

Solovchenko A, Verschoor AM, Jablonowski ND, Nedbal L (2016) Phosphorus from wastewater to crops: An alternative path involving microalgae. Biotechnol Adv 34(5):550–564

Suleiman AKA, Lourenço KS, Clark C, Luz RL, da Silva GHR, Vet LE, Cantarella H, Fernandes TV, Kuramae EE (2020) From toilet to agriculture: Fertilization with microalgal biomass from wastewater impacts the soil and rhizosphere active microbiomes, greenhouse gas emissions and plant growth. Resour Conserv Recycl 161:104924

Sutton C, Larsen S (1964) Pyrophosphate as a source of phosphorus for plants. Soil Sci 97(3):196–201

Turner BL, Cade-Menun BJ, Condron LM, Newman S (2005) Extraction of soil organic phosphorus. Talanta 66(2):294–306

Tyagi J, Mishra A, Kumari S, Singh S, Agarwal H, Pudake RN, Varma A, Joshi NC (2023) Deploying a microbial consortium of Serendipita indica, Rhizophagus intraradices, and Azotobacter chroococcum to boost drought tolerance in maize. Environ Exp Botany 206:105142

Varma A, Bakshi M, Lou B, Hartmann A, Oelmueller R (2012) Piriformospora indica: a novel plant growth-promoting mycorrhizal fungus. Agric Res 1:117–131

Verbruggen E, Rillig MC, Wehner J, Hegglin D, Wittwer R, van der Heijden MG (2014) Sebacinales, but not total root associated fungal communities, are affected by land-use intensity. New Phytol 203(4):1036–1040

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64(12):5004–5007

Vivienne H (2023) Urban wastewater treatment: Updating EU rules, EPRS: European Parliamentary Research Service. Belgium. Retrieved from https://policycommons.net/artifacts/3456816/urban-wastewater-treatment/4257250/ on 10 Jul 2024. CID: 20.500.12592/gcfmqp

Wang Y, Lambers H (2020) Root-released organic anions in response to low phosphorus availability: recent

progress, challenges and future perspectives. Plant Soil 447:135–156
Wang Z, Gui H, Luo Z, Zhen Z, Yan C, Xing B (2019) Dissolved organic phosphorus enhances arsenate bioaccumulation and biotransformation in Microcystis aeruginosa. Environ Pollut 252:1755–1763
Wang L, Amelung W, Willbold S (2021) 18O isotope labeling combined with 31P nuclear magnetic resonance spectroscopy for accurate quantification of hydrolyzable phosphorus species in environmental samples. Anal Chem 93(4):2018–2025
Wang X, Fan X, Chang W, Li K, Zhang M, Ping Y, Song F (2023) Combining the advantages of Rhizophagus intraradices and Serendipita indica to reduce the risk of Cd on soybean safety and environmental pollution. Rhizosphere 25:100669
Weiß M, Waller F, Zuccaro A, Selosse MA (2016) Sebacinales–one thousand and one interactions with land plants. New Phytol 211(1):20–40
Wickham H (2016) ggplot2: Elegant graphics for data analysis (Cham: Springer), 221–240. https://doi.org/10.1007/978-3-319-24277-4
Wilson JM, Trinick MJ (1983) Infection development and interactions between vesicular-arbuscular mycorrhizal fungi. New Phytol 93(4):543–553
Xie H, Li J, Zhu P, Peng C, Wang J, He H, Zhang X (2014) Long-term manure amendments enhance neutral sugar accumulation in bulk soil and particulate organic matter in a Mollisol. Soil Biol Biochem 78:45–53
Zhang L, Fan J, Ding X, He X, Zhang F, Feng G (2014) Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil. Soil Biol Biochem 74:177–183