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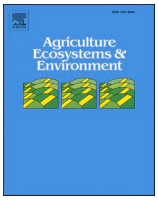

Effects of novel P fertilizers on microbial abundance related to N and P cycling in two on-farm systems

Stefanie Katharina Thaqi [a,b,*], Roberto Siani [b,c], Akane Chiba [b], Nora Vitow [d], Christel Baum [d], Peter Leinweber [d], Kerstin Panten [e], Michael Schloter [b,c], Stefanie Schulz [c]

[a] Technische Universität München, TUM School of Life Sciences, Chair of Crop Physiology, Alte Akademie 12, Freising 85354, Germany
[b] Technische Universität München, TUM School of Life Sciences, Chair of Environmental Microbiology, Emil-Ramann-Straße 2, Freising 85354, Germany
[c] Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Ingolstädter Landstraße 1, Neuherberg 85764, Germany
[d] University of Rostock, Chair of Soil Science, Justus-von-Liebig-Weg 6, Rostock 18059, Germany
[e] Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Crop and Soil Science, Bundesallee 58, Braunschweig 38116, Germany

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ABSTRACT

Phosphorus (P) is an essential macronutrient element for plant growth and development. Its limited availability makes alternative P sources crucial for fertilizer production. This study investigated the effects of three recycling-derived fertilizers with varying P solubility on microbial nutrient turnover at two fields in central Germany, Kiebitzbreite and Schmatzfelder Breite, which differ in management practices and soil characteristics. Samples were collected during the stem elongation stage of winter wheat from bulk soil and rhizosphere. Fertilization treatments included traditional triple superphosphate (TSP) and a no-P control (P0) for comparison. The abundance of microorganisms involved in P and Nitrogen (N) turnover was assessed by quantitative real-time PCR. Potential acid and alkaline phosphatase activity, mycorrhizal colonization rate, Carbon (C) to P, N to P ratios in the soil and the plant, and water-extractable P were measured. Although all treatments received the same amount of P, the differing solubilities of the fertilizers significantly affected water-extractable P levels, while nutrient ratios in the plant biomass remained comparable among sites and fertilizer treatments. However, the microbial strategies for maintaining P levels varied significantly across the sites. At the Kiebitzbreite, the site with silty loam texture and deep plowing, high ratios of available C and N to P in the soil were accompanied by high alkaline phosphatase activity and a larger abundance of arbuscular mycorrhizal fungi in the rhizosphere. Conversely, P solubilization was more pronounced at Schmatzfelder Breite, a site with finer soil texture managed by deep chiseling. Notably, the fertilization treatments influenced not only the abundance of bacteria catalyzing P turnover but also those catalyzing major steps of the N cycle, especially at Schmatzfelder Breite, where higher P solubility led to increased bacteria involved in N mineralization. This non-targeted effect on N cycling underscores the importance of fertilizer type, beyond just P supply, in influencing broader nutrient turnover dynamics. Our findings suggest that recycling-derived P fertilizers are promising alternatives to conventional P sources, though their on-farm impacts on microbial nutrient turnover vary significantly with site conditions and management.

1. Introduction

Phosphorus (P) is an indispensable, non-renewable macronutrient element essential for plant growth and development and is classified as a critical raw material (European Commission, 2020). Mineral P fertilizers are widely used to enhance low P levels in agricultural soils. However, the environmental impact of P mining and the finite nature of global P resources highlight the urgent need for sustainable alternatives (Chadwick et al., 1999; Walker and Syers, 1976). As global population increases, demand for P is expected to grow, intensifying the search for recycled P sources (Long et al., 2015). Recycling processes, including those that recover P from waste materials, offer sustainable alternatives, although these recycled fertilizers generally have lower solubility compared to conventional mineral fertilizers (Hertzberger et al., 2020; Raniro et al., 2022).

Among these recycled fertilizers, struvite, sewage sludge ash, and

* Correspondence to: Ingolstädter Landstraße 1, Neuherberg 85764, Germany.
E-mail address: stefanie.thaqi@tum.de (S.K. Thaqi).

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bone char are emerging as promising sustainable options for agricultural applications. Struvite, recovered from wastewater, and SSA, produced through sewage sludge incineration, allow nutrient recycling from urban and industrial waste stream (Heyl et al., 2023; Ohtake and Tsuneda, 2019). Bone char, is derived from pyrolyzed animal bones and offers distinct benefits, including high P and calcium (Ca) content. However, bone char's agronomic effectiveness varies with soil and crop type, necessitating further research to confirm its benefits under diverse conditions (Leinweber et al., 2019). Due to economic and practical challenges, as well as a need for field-based evidence on long-term impacts, large-scale application of these recycled P sources is still limited (Heyl et al., 2023; Leinweber et al., 2019).

The soil microbiome plays an essential role in mobilizing and transforming P into plant-available forms (Richardson and Simpson, 2011), which can help to address these limitations. For instance, sulfur (S)-enriched bone char relies on microbial oxidation of sulfur for P release (Zimmer et al., 2019). Research indicates that recycled P fertilizers affect soil microbial communities differently from mineral fertilizers (Grafe et al., 2021; Robles-Aguilar et al., 2020). Mineral fertilizers, such as triple super phosphate (TSP), are known for their rapid P release, which has been linked to reduced microbial P turnover and lower abundance of bacteria harboring the *phoD* gene that codes for alkaline phosphatase (ALP) (Chen et al., 2019; Wei et al., 2019). The reduced abundance of microbes that carry a specific function is often linked to significantly reduced microbial activity and subsequent negative impacts on crop yields (Dai et al., 2017). Additionally, mineral N and P fertilizers can decrease mycorrhizal colonization in roots (Wang et al., 2020), negatively affecting nutrient uptake and plant health (Baum et al., 2015; Bowles et al., 2016). In contrast, slow-releasing P fertilizers, like struvite and phosphate rock, offer benefits for the soil microbiome, increasing microbial biomass (Teng et al., 2018), enhancing diversity (Chen et al., 2022; Hayatsu, 2014), and more activity in nutrient turnover (Liao et al., 2020). Such fertilizers are also linked to higher arbuscular mycorrhizal fungi (AMF) colonization (Alloush and Clark, 2001; Van Geel et al., 2016), which can enrich the rhizosphere bacterial community through additional carbon (C) release (Changey et al., 2019; Xu et al., 2018; Zhang et al., 2016). Moreover, organic C, N, and P further exhibit co-metabolic interactions. For example, the Pho regulon in bacteria controls inorganic P metabolism. It interacts with N and C regulatory systems, influencing bacterial growth and metabolism (Santos-Beneit, 2015) - this further influences microbial interactions and nutrient cycling within the rhizosphere.

The maintenance of stable nutrient ratios is a crucial factor influencing the activity of microorganisms (Cleveland and Liptzin, 2007). The ability to solubilize P from slow-release fertilizers is not only dependent on the type of fertilizer but also on soil nutrient stoichiometry. Elevated nutrient stoichiometry has been found to promote bacteria harboring P-associated genes such as *phoD* involved in organic P mineralization, and *pstS*, which encodes a high-affinity phosphate--binding protein that facilitates inorganic phosphate uptake, thereby influencing the soil microbial community (Bergkemper et al., 2016). This understanding of soil nutrient stoichiometry provides valuable insights into the factors influencing soil health and the promotion of beneficial bacteria. Luo et al. (2019) showed that under slow-releasing P fertilizers, microorganisms may experience P limitation in the rhizosphere due to competition with plants for P. However, studies have shown that slow-release P fertilizers, such as struvite and phosphate rock, have the potential to overcome this limitation. This was observed in studies where P recycling, indicated by *phoD* harboring bacteria abundance, peaked in plots with no or slow-release P fertilization, highlighting its potential for supporting sustainable farming practices (Grafe et al., 2021).

Tillage, much like fertilization, plays a significant role in shaping the structure of soil microbial communities. Reduced tillage has been associated with higher rates of mycorrhizal root colonization and increased activity in the denitrifying community, thereby creating a supportive environment for microbial processes (Bowles et al., 2017; Schmidt et al., 2019). Most studies have investigated these dynamics under controlled conditions, often needing more complexity in real farm settings where soil properties, crop rotations, and tillage practices interact. To address these gaps, we conducted field experiments at two sites with differing soil textures, crop rotations, and management practices to understand the influence of alternative P fertilization on the soil microbiome. We hypothesize that (1) differences in P-fertilizer solubility will directly affect bacteria involved in P transformation and alter the abundance of bacteria involved in N turnover. (2) Beyond the general effects of P fertilization, we hypothesize that site-specific differences in soil characteristics, management practices (e.g., deep chisel tillage versus plowing), and environmental conditions will modulate this response. Thus, the magnitude and nature of these effects are expected to vary between sites, reflecting the interplay between fertilization treatments and local conditions.

2. Materials and methods

2.1. Field sites and experimental design

Two on-farm field experiments were set up in 2016 at Kiebitzbreite (KB; 51°55'N, 10°39'E, 206 m a.s.l.) and Schmatzfelder Breite (SB; 51°53'N, 10°47'E, 205 m a.s.l.) in Saxony-Anhalt, Germany. Despite their proximity and comparable climate (annual precipitation 608 mm, mean temperature 9.5 °C; 1991–2020; German Weather Service, Fig. S1), the sites differed in soil type, crop rotation, tillage practices, and fertilization rates (Table S1). Before the experiment, SB received 44 kg P ha^{-1} as $(NH_4)_2HPO_4$ (diammonium phosphate) in May 2015 and 25 t ha^{-1} pig manure in October 2014, while KB was treated with an unknown amount of compost during the 2014/2015 period. In 2016, winter barley was cultivated at KB and winter wheat at SB.

In a randomized block design with four replicates (Fig. S2), experimental P fertilizers were applied annually from 2016–2018 before seeding. Since 2016, these fertilizers served as the sole P source and were manually applied shortly before seeding to ensure shallow incorporation during the seeding process. Application amounts were calculated based on the total P concentration of the individual fertilizer. Annually, P application rates were calculated based on the farmers' anticipated crop yield and available soil P test results according to the fertilization guidelines from Saxony-Anhalt (LLFG, 2008). At KB, rates were 41.2 kg P ha^{-1} in 2017, 36.25 kg P ha^{-1} in 2018, and 34.5 kg P ha^{-1} in 2019. At SB, application rates were slightly higher, with 39.75 kg P ha^{-1} in 2017, 41.5 kg P ha^{-1} in 2018, and 45.1 kg P ha^{-1} in 2019.The treatments included three recycling fertilizers, a control (P0), and a mineral fertilizer (TSP). BC^{plus} was produced by pyrolyzing defatted, degelatinized animal bone chips at 600–800 °C (LeGeros, 2017), followed by sulfur (S) surface modification to enhance its solubility and improve P availability for plants ((Zimmer et al., 2019); patent DE102011010525). The solubility of BC^{plus}, as well as the other fertilizers, was evaluated using three chemical extraction methods: P_{water} (water-soluble P fraction), neutral ammonium citrate (NAC), and citric acid (CA) (European Union, 2003). These solubility fractions were measured calorimetrically at 882 nm following the method of John (1970). BC^{plus} exhibited the lowest solubility among the tested fertilizers, with 1 % P_{water}, 39 % NAC, and 79 % CA. Ash, a thermochemically treated sewage sludge ash, was produced by combining Ca phosphate-rich sewage sludge ash with sodium salts (Na_2SO_4) and calcining the mixture under reducing conditions. This process increases phosphorus solubility by forming plant-available P-containing mineral phases while reducing heavy metal content (Stemann et al., 2015). Ash exhibited intermediate solubility, with 2 % P_{water}, 86 % NAC, and 93 % CA. Struvite (Str) was derived from phosphorus recovery in the Airprex® wastewater treatment process in Berlin-Waßmannsdorf (Berliner Wasserbetriebe, Germany). P was precipitated as magnesium ammonium phosphate (MAP) by stripping CO_2 from the sludge and adding

magnesium salts, producing a fertilizer suitable for plant use ((Kratz et al., 2019); CNP Cycles GmbH, Germany) with relatively high solubility (2 % P_{water}, 99 % NAC, and 98 % CA). TSP, a highly soluble mineral fertilizer, was included as a reference treatment. Its solubility far exceeded the recycled fertilizers, with 87 % P_{water}, 96 % NAC, and 101 % CA, making it immediately available for plant uptake.

2.2. Sampling and sample preparation

Sampling was conducted in May during the shoot elongation stage according to morphological traits (BBCH 31/32) when P demand for wheat was highest (Römer and Schilling, 1986), and high P-solubilizer abundance was previously observed in BC^{plus} treatment (Grafe et al., 2021). During the 2018/2019 vegetation period, when *Triticum aestivum* (cultivar Kredo at KB and Julius at SB) was cultivated at both sites, dry matter (DM) plant biomass at sampling time ranged from 2.6 (P0) to 4.9 (Str) DM t ha^{-1} in KB and from 4.0 (P0) to 5.0 (Str) DM t ha^{-1} in SB (Table S2).

Rhizosphere and bulk soil samples were collected from each plot, totaling 240 samples across two fields, five fertilization treatments, two soil compartments, four replicates, and three pseudo replicates. Three plants per plot were excavated to sample rhizosphere soil firmly attached to the roots after shaking. Bulk soil (0–10 cm depth) was collected from a spot 10 cm away from these plants. All soil samples were sieved to < 2 mm. Rhizosphere and bulk soil samples were either immediately frozen for DNA extraction or stored at 4°C for chemical analyses, including total dissolved nitrogen (TDN), dissolved organic nitrogen (DON), ammonium (NH_4^+), nitrate (NO_3^-), pH, and dissolved carbon (DC). A composite bulk soil sample of ten soil cores per plot (0–10 cm) was further prepared to assess Ca acetate lactate-extractable P (P_{CAL}), P_{water}, ALP, and acid phosphatase (ACP). For phosphatase analyses, the samples were stored at 4 °C; for P_{CAL} and P_{water} analysis, the soil was air-dried and sieved. Additionally, four 0.25 m^2 sections per plot were cut and combined for plant biomass determination and plant nutrient analysis of C, N, and P contents. Samples were dried at 60 °C in a ventilated oven until constant weight and ground to < 0.5 mm using an ultracentrifugal mill (Retsch ZM 200, Haan, Germany). Roots from five plants were used separately to assess mycorrhizal colonization. An overview of sample storage and processing methods is available in Table S3.

2.3. Chemical analysis, soil enzyme activities, and mycorrhizal colonization

Soil water content was determined gravimetrically by weighing 2 g of each sample before and after drying at 65 °C for 48 h in aluminum boats. The pH (1:10 soil: 0.01 M $CaCl_2$ ratio), DC, TDN, DON, NH_4^+, and NO_3^- (1:4 soil: 0.01 M $CaCl_2$ ratio) concentrations were determined using standard methods (DIN ISO 10390; DIN ISO 10694; DIN ISO 14256-2) with a pH meter (WTW InoLab Level 1, Weilheim, Germany), DIMA-TOC 2000 + DIMA-N (Dima Tec, Langenhagen, Germany) or a Skalar (Skalar Analytical B.V., Breda, Netherlands) respectively. P_{CAL} was extracted with Ca acetate lactate (Schüller, 1969), and P_{water} was extracted following a slightly modified protocol from van der Paauw et al. (1971), using 1.5 g soil and 70 mL of distilled H_2O. P in both extracts was quantified colorimetrically, based on the intensity of the blue color formed in the reaction (Specord 50, Analytik Jena, Germany) using the molybdenum blue method (Murphy and Riley, 1962).

P concentrations in plant samples were determined after microwave-assisted digestion with 6 mL nitric acid and 1.5 mL hydrogen peroxide (CEM MARS, Metthews, NC, USA) using ICP-OES (icap 6000, Thermo Fisher, Cambridge, United Kingdom) at a wavelength of 177.4 nm. Total C and N in plant material were measured using approximately 30 mg sample material with an Elemental Analyzer (Euro EA, Eurovector, Italy).

The potential activity of ACP and ALP was analyzed following Schinner et al. (1991) and using the method established by Eivazi and Tabatabai (1977) and Tabatabai and Bremner (1969), with colorimetric analysis of the filtrate at 400 nm (Specord 50, Analytik Jena, Germany).

The ratios of plant-available soil nutrients were calculated by dividing DC or TDN by P_{CAL}. Plant material's total C, N, and P ratios were calculated from total concentrations.

Roots were washed with distilled water to quantify mycorrhizal colonization and cut into 10 mm segments. These segments were treated with 10 % KOH for 24 h at room temperature, acidified with 1 % HCl for 15 min, and stained with 0.05 % chlorazol black E for 24 h (Brundrett et al., 1984). AMF colonization was quantified using the intersection method (McGonigle et al., 1990).

2.4. DNA extraction

DNA was extracted from 0.30 g of soil and first homogenized in Lysing Matrix Tubes E (MP Biomedicals, USA) using a Precellys24 (Bertin Technologies, France). Extraction followed a phenol-chloroform-based protocol (Lueders et al., 2004; Stempfhuber et al., 2017), with DAN stored at −20 °C. A negative control was included by performing the extraction without adding soil. DNA quality was determined by measuring absorption ratios of 260 nm/280 nm and 260 nm/230 nm using a photometer (Nanodrop ND-1000; Thermo Fischer Scientific, Ma, USA), and the quantity of total genomic DNA was determined using the Quant-IT™ Pico-Green® dsDNA Assay Kit (Thermo Fischer Scientific, MA, USA).

2.5. Quantitative polymerase chain reaction (qPCR) measurement

Real-time quantitative PCR was performed on the 7300 Real-Time PCR System (Applied Biosystems, Germany) with SYBR Green® as a fluorescent dye. Each 25 µL reaction contained 12.5 µL of SYBR Green® (Thermo Fisher Scientific, USA), forward and reverse primers (Metabion, Germany), 0.5 µL BSA (3 %, Sigma, Germany), and DEPC-treated water. Details of marker genes, primers, thermal profiles, and calibration standards are provided in the Table 1 and S4.

Each qPCR assay consisted of 40 cycles, with conditions tailored to each target gene. To ensure reliability and adherence to best practices, we followed the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines (Bustin et al., 2009). Prior to performing qPCR, a dilution test was conducted for each sample to exclude potential inhibition, with the optimal sample dilution

Table 1
Overview of the marker genes to detect the abundance of microorganisms catalyzing important nutrient cycling processes.

Processes	Gene		Function
N mineralization	*apr*	Alkaline metallopeptidases	Organic N → NH_4^+
	chiA	chitinase A	
N fixation	*nifH*	The iron protein of nitrogenase	N_2 -> NH_4^+
Ammonia oxidation	AOA-*amoA*	Ammonia monooxygenase alpha subunit of archaea	NH_4^+ → H_3NO
	AOB-*amoA*	Ammonia monooxygenase alpha subunit of bacteria	
Nitrite oxidation	*nxrA*	Alpha subunit of nitrite oxidoreductase	NO_2- → NO_3^-
Denitrification	*nirK*	Cu-containing nitrite reductase	NO → N_2O
	nirS	Cytochrome cd1-containing nitrite reductase	
	nosZ	Nitrous oxide reductase	N_2O → N_2
P solubilization	*gcd*	Glucose dehydrogenase	Inorganic P → PO_4^{3-}
P mineralization	*phoD*	Alkaline phosphatase	Organic P → PO_4^{3-}
P uptake	*pitA*	Low-affinity P transport system	
	pstS	High-affinity P transport system (periplasmic phosphate-binding protein)	

determined as 1:64 based on preliminary tests (data not shown). Each run included a reference dilution series of the respective gene, derived from a bacterial culture cloned into a suitable vector system, alongside no-template controls. Quality control measures included melting curve analysis to confirm amplification specificity and verification of product size by 1.5 % agarose gel electrophoresis. Amplification efficiencies were calculated as $Eff_{slope} = 10^{-1/slope}$ x 100 and ranged from 78–108 %, consistently achieving r^2 values above 0.99. These efficiencies align well with those reported in other studies (Harris et al., 2021; Kurth et al., 2021; Stempfhuber et al., 2017). Samples with fewer than 10 copies μL^{-1} were excluded from the analysis (set as NA).

2.6. Statistical analysis

Data analysis was performed in R Studio (v4.3.1) (R Core Team, 2023). A linear mixed-effect model was applied to log-transformed data for each soil compartment to analyze the soil and plant properties, with fertilization treatment and site as fixed factors and plot as a random effect to account for variability across plots. This approach is well-suited to our randomized block design, allowing for the analysis of fixed effects while controlling for plot-level variation, thereby enhancing the generalizability of the results. The model structure was nlme::lme(variable of interest ~ fertilizer * site, random = ~1 | plot). Subsequently, the output from this model was used in a two-way ANOVA to evaluate the significance of fixed factors and their interaction. Pairwise comparisons between fertilization treatments within each site were performed using Tukey's Post-Hoc (*emmeans*, v1.8.8) (Lenth et al., 2018) to identify differences specifically between treatments.

For gene abundance data, a similar linear mixed-effects model was applied, but for post-hoc testing, the marginal effects approach (*marginaleffects*, v0.15.1) (Arel-Bundock, 2023) was used. This method estimates the expected value of the dependent variable for each level of the predictor variable while holding other covariates constant, providing robust estimates even in the presence of variability and deviations from normality, as in the case of gene abundance data.

Plots were created using *ggplot2* (v3.4.4) (Wickham, 2016). Nonmetric multidimensional scaling (NMDS) analysis based on the Bray-Curtis dissimilarity was performed to visualize the abundance of microbial functional groups among different fertilization treatments, soil compartments, and sites. Significant differences were evaluated using permutational multivariate analysis of variance (PERMANOVA) with the adonis functions from the *vegan* (v2.6–4) package (Oksanen et al., 2022).

3. Results

3.1. Soil and plant chemical properties

Grain yield was significantly affected by fertilization ($p = 0.02$), but post-hoc testing revealed no significant pairwise differences between fertilizer treatments within sites (Table 2). Across the sites, grain yields varied between 5.8 DM t ha^{-1} for the control treatment (P0) and 7.4 DM t ha^{-1} for TSP at KB, and between 6.4 DM t ha^{-1} for P0 and 8.0 DM t ha^{-1} for Str at SB (Table S2). At both sites, nutrient ratios in soil and plants did not differ significantly across fertilization treatments (Fig. 1). P_{water} levels varied notably by site and treatment, with overall lower values at KB compared to SB. At KB, the TSP treatment resulted in significantly higher P_{water} (8.6 mg kg^{-1} dry weight (dwt); $p = 0.01$) compared to BC^{plus} (2.4 mg kg^{-1} dwt), as did the Str treatment (8.4 mg kg^{-1} dwt; $p = 0.004$). Additionally, P_{water} in the Str treatment was significantly higher than in P0 (2.47 mg kg^{-1} dwt; $p = 0.05$). At SB, P_{water} levels in BC^{plus} and P0 (both 7.6 mg kg^{-1} dwt) were significantly lower compared to other treatments. Specifically, Ash showed higher P_{water} (16.1 mg kg^{-1} dwt), significantly exceeding BC^{plus} ($p = 0.01$) and P0 ($p = 0.005$). The Str treatment had the highest P_{water} at 24.7 mg kg^{-1} dwt, which was significantly higher than both BC^{plus} ($p = 0.001$) and P0 ($p < 0.001$). TSP also had elevated P_{water} (16.1 mg kg^{-1} dwt) compared to BC^{plus} ($p = 0.01$) and P0 ($p = 0.006$) (Table 2 and S5).

Due to limited rhizosphere soil material, only pH, DC, DON, NH_4^+, and NO_3^- were measured as indicators of root exudation and N availability. DC and NO_3^- concentrations in bulk soil and rhizosphere were unaffected by fertilization at both sites (Table 2). Significant differences in DON were found only in the rhizosphere at SB, where P0 (12.3 mg kg^{-1} dwt; $p = 0.04$) was significantly higher than Str (7.0 mg kg^{-1} dwt) (Table 2 and S5). NH_4^+ levels at SB varied significantly in both soil compartments. In the bulk soil, Str (0.6 mg kg^{-1} dwt) had lower concentrations than BC^{plus} (1.0 mg kg^{-1} dwt; $p < 0.001$), and in the rhizosphere, NH_4^+ concentrations were significantly lower for Str

Table 2
Significant results ($p < 0.05$) for at least one fixed factor in the ANOVA analysis on linear mixed-effect models. Significant values are shown in italics.

	Bulk Soil			**Rhizosphere or Plant**		
	Fertilizer	Site	Fertilizer:Site	Fertilizer	Site	Fertilizer:Site
16 S rRNA	0.27	*< 0.01*	0.35	0.31	*< 0.01*	0.75
AMF	0.69	*< 0.01*	0.29	0.61	*< 0.01*	*< 0.01*
gcd	0.97	*< 0.01*	0.61	0.92	*< 0.01*	0.31
phoD	*0.02*	*< 0.01*	0.08	0.59	*< 0.01*	0.42
pitA	0.45	*< 0.01*	0.75	0.13	0.34	0.92
pstS	0.75	*< 0.01*	0.82	0.69	*< 0.01*	0.99
apr	0.99	*< 0.01*	0.99	*< 0.01*	*< 0.01*	0.21
chiA	0.77	*< 0.01*	0.71	0.92	*< 0.01*	0.09
AOA	*< 0.01*	*< 0.01*	0.38	*0.06*	*0.02*	0.61
AOB	0.35	*0.01*	0.74	*0.01*	*< 0.01*	0.35
nxrA	0.69	*< 0.01*	0.48	0.36	*< 0.01*	0.15
nirK	0.26	0.30	0.62	*< 0.01*	*0.03*	0.72
nirS	0.49	*< 0.01*	0.69	0.59	*< 0.01*	*0.01*
nosZ	0.45	*< 0.01*	0.69	0.61	*0.01*	0.51
pH	*< 0.01*	*< 0.01*	*0.02*	0.16	*< 0.01*	*< 0.01*
DC	*0.03*	*< 0.01*	0.18	*0.02*	*< 0.01*	0.07
DON	*0.04*	1.00	1.00	0.14	1.00	0.49
NO_3^-	0.42	*< 0.01*	*< 0.01*	0.71	0.14	0.98
ALP	*< 0.01*	*< 0.01*	*< 0.01*			
ACP	0.16	*0.01*	0.10			
P_{water}	*< 0.01*	0.25	0.65			
DC:P_{CAL} soil	0.93	*0.01*	0.23			
C:P plant				0.35	*< 0.01*	0.4
Grain yield				*0.02*	0.14	0.43

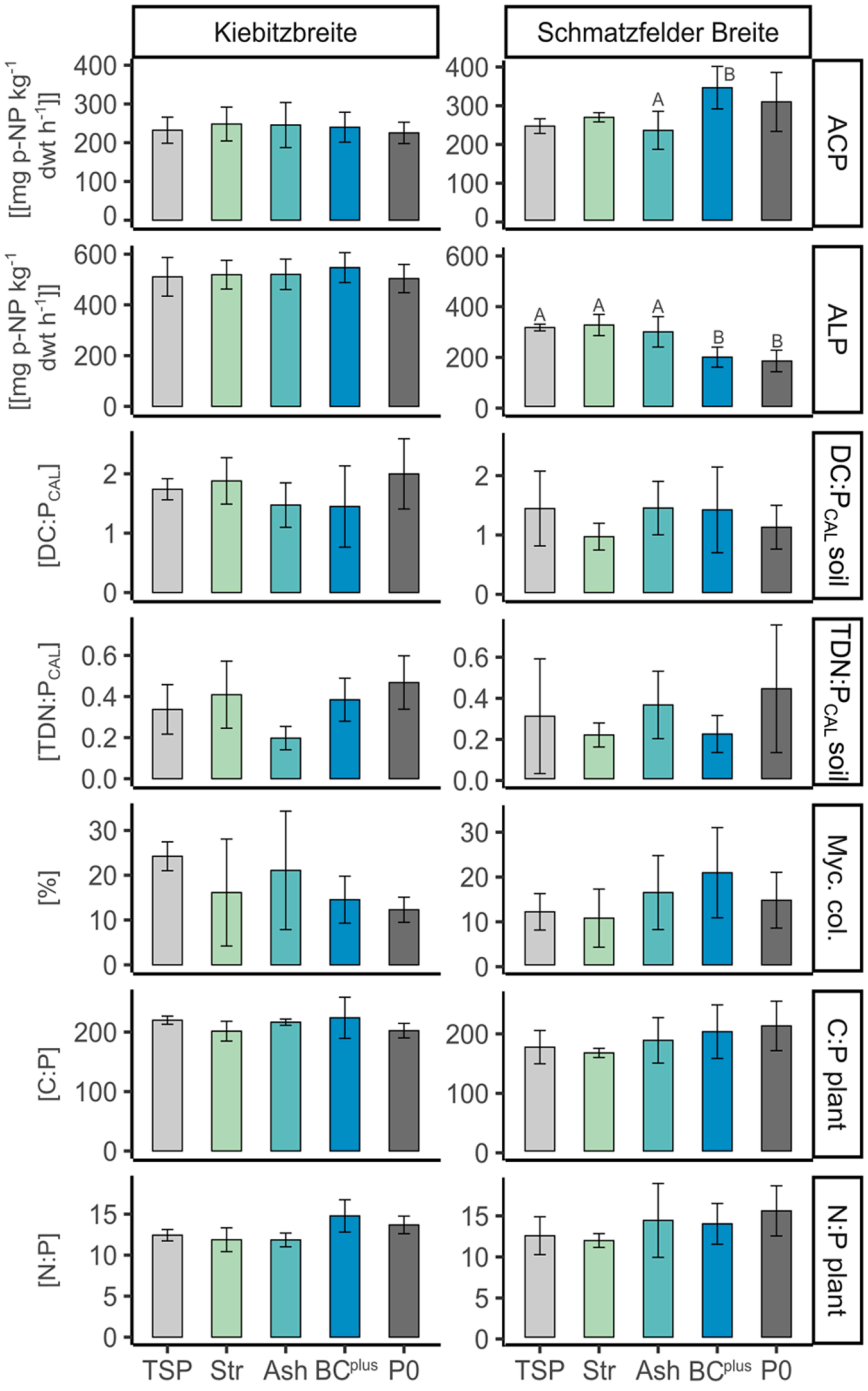

Fig. 1. Bar plots of the chemical properties of the soil and the plant. A mixed sample was taken from each site per plot and fertilization treatment (n = 4). The significant differences ($p < 0.05$) between treatments within one site are indicated with capital letters. The facets display the potential enzyme activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) in bulk soil [mg p-NP kg^{-1} dry weight (dwt) h^{-1}], DC:P$_{CAL}$ and TDN:P$_{CAL}$ ratios of plant-available nutrients in bulk soil [based on mg kg^{-1} dwt], the percentage of mycorrhizal colonization (Myc. col.) in plant roots [%], and the C:P and N:P ratios in plant tissues [based on mg kg^{-1} dwt].

(0.8 mg kg^{-1} dwt) compared to Ash (1.6 mg kg^{-1} dwt; $p = 0.001$) and P0 (1.3 mg kg^{-1} dwt, $p = 0.03$). At KB, no significant effects of fertilization on NH_4^+ concentrations was observed (Table 2 and S5).

Although significant pH differences were observed in the rhizosphere at KB and in the bulk soil at SB (Table 2), these differences were marginal (Table S5) and are unlikely to have a meaningful effect on soil processes or plant soil interactions. Generally, pH was higher at KB (6.9–7.1) compared to SB (6.4–6.7)

3.2. Potential enzyme activities

ALP was generally higher in KB, whereas ACP was higher in SB, corresponding with the lower pH levels observed at SB (Fig. 1 and Table 2). At SB, ALP concentration were significantly lower in the BC^{plus} (193.9 mg-NP kg^{-1} dwt^{-1}) and P0 (178.8 mg-NP kg^{-1} dwt^{-1}) treatments compared to the other treatments. Specifically, ALP in Ash (293.7 mg-NP kg^{-1} dwt) was significantly higher than BC^{plus} ($p = 0.002$) and P0 ($p < 0.001$). ALP in the Str treatment (320.5 mg-NP kg^{-1} dwt) was

significantly higher than both BC^{plus} ($p < 0.001$) and P0 ($p < 0.001$), while TSP also exhibited higher ALP (310.7 mg-NP kg^{-1} dwt) compared to BC^{plus} ($p < 0.001$) and P0 ($p < 0.001$). Regarding ACP, Ash (231.8 mg-NP kg^{-1} dwt^{-1}) was significantly lower than BC^{plus} (342.0 mg-NP kg^{-1} dwt^{-1}; $p = 0.02$).

3.3. Mycorrhizal colonization and AMF abundance

Fertilization did not significantly affect mycorrhizal colonization or AMF abundance at either site (Table 2). However, trends differed between the sites.

At KB, mycorrhizal colonization was highest in the TSP treatment, followed by Ash, Str, BC^{plus}, and lowest in P0 (Fig. 1 and S3). AMF abundance in the rhizosphere showed a different trend, with the highest levels in BC^{plus}, followed by Str, Ash, and P0, and the lowest in TSP. In the bulk soil at KB, AMF abundance was highest in P0, with Ash, Str, BC^{plus}, and TSP showing progressively lower levels.

At SB, trends in mycorrhizal colonization and AMF abundance were more consistent (Fig. 1 and S3). Mycorrhizal colonization peaked in BC^{plus}, followed by Ash, P0, TSP, and lowest in Str. Rhizosphere AMF abundance was highest in TSP, followed by Str, BC^{plus}, and Ash, with the lowest levels in P0. P0 again had the highest AMF abundance in the bulk soil, followed by Ash, BC^{plus}, and TSP, with the lowest levels observed in Str.

3.4. Abundance of bacterial communities catalyzing critical processes of N and P turnover

Gene copy numbers were related to gram dry weight soil as the bacterial biomass did not differ significantly among the samples (Table 2). Nonmetric multidimensional scaling (NMDS) and PERMANOVA analyses revealed that the soil compartment explained the greatest variance in the bacterial community (46 %, $p < 0.001$), followed by the site (10 %, $p < 0.001$), while fertilization treatment explained a minor portion (1 %, $p = 0.03$) (Fig. 2, Table S6). Notably, clustering by site was more pronounced in the rhizosphere than in bulk soil.

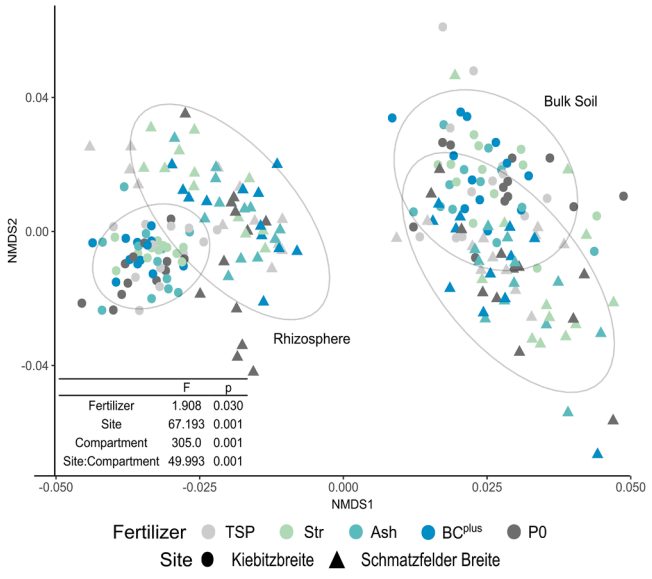

Fig. 2. Nonmetric multidimensional scaling (NMDS) plot, showing 95 % confidence interval ellipses for soil compartments at both sites. The NMDS analysis was performed on microbial abundances across fertilization treatments, soil compartments, and sites. Sites are represented by symbols (Kiebitzbreite = circle, Schmatzfelder Breite = triangle), and fertilization treatments are distinguished by color. PERMANOVA *p*-values and F-values for significant comparisons are displayed, with full results in Table S6 of the Supplementary Materials.

soil.

The effect of the soil compartment was primarily driven by differences in the abundance of bacteria harboring *phoD* and *pstS*, which were 20 times more abundant in the rhizosphere than in bulk soil. Conversely, bacteria carrying the *nirK* gene (copper-containing nitrite reductase) were around five times less abundant in the rhizosphere (Fig. S4). At KB, bacteria that code for the *nosZ* gene (nitrous oxide reductase) and archaea carrying the *amoA* (AOA, ammonia monooxygenase) were at least five times less abundant in the rhizosphere compared to bulk soil. At SB, bacteria harboring the *apr* gene (alkaline metallopeptidase) were more abundant in the rhizosphere. Interestingly, bacteria with the *apr* gene tended to be more abundant in treatments with more soluble P fertilizers.

The fertilization effects were distinct between bulk soil and rhizosphere, with separate analyses revealing stronger fertilization effects in the rhizosphere, especially at SB (Fig. 2). To visualize these effects, ratios of gene copy numbers in fertilized versus control treatments were calculated for each site and compartment (Fig. 3). In the rhizosphere at SB, fertilization increased the abundance of bacteria involved in N turnover, particularly for *nxrA* (alpha subunit of nitrite oxidoreductase), AOA, *amoA* carrying ammonia-oxidizing bacteria (AOB), and *apr*. All fertilization treatments showed higher abundances of these genes than P0, with significant differences observed in at least one comparison within a given site and compartment (Fig. 3 and S3, Table 2). This was most pronounced for *apr*, which was 8 and 12 times higher in the TSP and Str treatments than in P0. At KB, similar trends were observed but less pronounced. Significant differences were only observed for bacteria harboring the *nirS* gene (cytochrome cd1 nitrite reductase) with higher gene copy numbers in TSP (1.4×10^7 gene copies g^{-1} dwt) compared to Ash (1.1×10^7 gene copies g^{-1} dwt).

In bulk soil, only two genes showed significant responses to fertilization. At KB, the abundance of AOA was two times lower in Ash (1.1×10^7 gene copies g^{-1} dwt) compared to the control (2.6×10^7 gene copies g^{-1} dwt). AOA levels in Ash were also lower than in BC^{plus} (4.3×10^7 gene copies g^{-1} dwt) and TSP (3.3×10^7 gene copies g^{-1} dwt). At SB, fertilization significantly affected *phoD* carrying bacteria, which were less abundant in Str (4.6×10^6 gene copies g^{-1} dwt) and Ash (3.4×10^6 gene copies g^{-1} dwt) treatments compared to TSP (8.8×10^6 gene copies g^{-1} dwt).

4. Discussion

4.1. Soil management and P availability shape P mobilization

Our study found no significant changes in plant C:P and N:P ratio across fertilization treatments, suggesting relatively stable nutrient acquisition at both sites (Fig. 1, Table S5). However, P_{water} levels were consistently lower at KB compared to SB (Fig. 1, Table S5). In P-limited conditions, plants often form symbioses with AMF (Corradi and Bonfante, 2012) or stimulate microbial activity in the soil (Sun et al., 2022). Distinct P mobilization strategies emerged between the sites. At KB, higher AMF abundance in the rhizosphere, especially in BC^{plus} and P0 treatments (Fig. S4), suggests a potential response to lower P_{water} or the gradual P release from BC^{plus}. This observation aligns with Van Geel et al. (2016), who found that slow-release fertilizers can enhance AMF diversity. Interestingly, despite higher AMF abundance at KB, this was not accompanied by increased mycorrhizal root colonization (Fig. 1). Conversely, at SB, mycorrhizal colonization was highest under low soluble fertilizers. This trend may reflect site-specific differences in soil management practices and textures, as deep chisel tillage at SB, likely preserved AMF mycelium and spores, thereby promoting root colonization and influencing microbial structure. Such reduced soil disturbance is known to support AMF development and enhance rhizosphere conditions (Bowles et al., 2017; Schmidt et al., 2019). High P availability reduces plant reliance on AMF, shifting nutrient uptake towards direct acquisition (Lambers et al., 2015; Werner and Kiers, 2015). This may

Fig. 3. Heatmap illustrating the ratio between gene copy numbers in the different fertilization treatments. Average gene abundances were calculated for each treatment (TSP, Str, Ash, BC, P0) and normalized to the control treatment (P0). When the values of the treatments were higher than those of the control treatment, this ratio was calculated as r = treatment/control. Conversely, when the treatment values were lower, it was computed as r = -control/treatment. Values with r > 1 indicate higher gene abundances in the fertilization treatment and are represented in shades of red. Values with r < -1 indicate higher abundances in the control treatment and are color-coded on a blue scale. Only values exceeding 1.25 or falling below −1.25 are additionally displayed as numerical values.

explain the generally lower AMF abundance at SB, where P_{water} levels were higher, compared to KB, where AMF likely contributed more to plant P acquisition.

AMF may enhance ALP activity in soils, either directly through phosphatase production or indirectly by stimulating microbial phosphatase activity (Aono et al., 2004; Feng et al., 2007). This potentially explain the generally higher ALP levels observed at KB (Fig. 1 and S3). Simultaneous increases in AMF abundance, *phoD* harboring bacteria, and ALP activity at KB align with findings from low-P soils (Masrahi et al., 2020). Additionally, higher pH levels at KB may enhance ALP activity, as it performs better in alkaline conditions (Dick et al., 2000). Beyond P mobilization, AMF also support microbial diversity and ecosystem stability by facilitating nutrient exchange and secreting enzymes and organic acids (Bogati and Walczak, 2022; Noceto et al., 2021; Xu et al., 2018; Zhang et al., 2024). Consistent with these observations, the abundance of bacteria harboring the *phoD* gene was generally higher at KB (Fig. S3, Table S5), indicating microbial adaptation to lower P_{water} levels at this site.

In contrast, P mobilization in the rhizosphere of SB was characterized by a higher abundance of *gcd* (glucose dehydrogenase) harboring bacteria. Higher P_{water} levels at SB suggest that recycling organic P may be less critical at this site. These findings align with metagenomic studies showing increased solubilization potential in P-rich soils, while Pho regulon genes dominate under P limitation (Bergkemper et al., 2016). These observations highlight the crucial role of microbial adaptations, including phosphatases and high-affinity phosphate transporters, in sustaining nutrient cycling in nutrient-depleted environments, underscoring the importance of microbial responses to soil nutrient stoichiometry.

4.2. *Changes in soil nutrient stoichiometry alter N turnover processes*

Soil nutrient stoichiometry strongly drives microbial turnover as microorganisms adjust to maintain stable macronutrient ratios (Cleveland and Liptzin, 2007). In our study, although N fertilization was similar at both sites, differences in P_{water} levels were evident, reflecting differences in P fertilizer solubility (Table 2 and S5). The lower P_{water} levels at KB may be linked to its sandier soil, which generally retains N and P less effectively than loamy soils (Amberger, 1996; Augusto et al., 2010; Harris et al., 1994). Additionally, sand content often serves as a proxy for quartz content in soils (Bui and Henderson, 2013), a mineral that tends to be low in P (Hahm et al., 2014; Vitousek et al., 2010). The ratios of available soil nutrients were generally higher at KB (Fig. 1, Table 2 and S5), which may reflect differences in soil composition and nutrient retention across the sites. Our data indicated that microbial N cycling was more responsive to fertilizer treatments than P cycling, with notably stronger effects observed at SB. This suggests that the less sandy soil conditions and higher P_{water} levels at SB may enhance microbial responsiveness to N inputs. In general, the strongest effects were observed for AOB and bacteria carrying the *apr* gene. The high abundance of AOA and AOB in the Str treatment corresponded with the additional NH_4^+ input, as lower NH_4^+ levels in the soil suggest rapid consumption by these ammonia oxidizers. Bacteria possessing the *apr* gene were particularly abundant in Str and TSP treatments, suggesting

that the higher solubility of these P fertilizers may promote both ammonia oxidizer abundance and N mineralization. Previous studies have established a connection between P and N turnover. For instance, bioavailable P fractions in soil can enhance N fixation (Bergkemper et al., 2016), and P deficiency has been linked to reduced N turnover (Cui et al., 2020; Santos-Beneit, 2015). This relationship may also explain the decline in N cycling microorganisms under the P0 treatment at SB, while accounting for the generally lower treatment effects observed at KB. Site-specific conditions, including tillage practices may also contribute to these differences. Plowing at KB may have diminished treatment effects compared to deep chisel tillage at SB, as evidence suggests that less intensive soil management can positively influence soil biological activity compared to conventional plowing systems (Sándor et al., 2020). Our Study indicates that N turnover processes may be strongly influenced by local soil and management factors rather than by fertilization treatments alone. This underscores the need for a holistic approach to understanding soil nutrient dynamics.

4.3. Plant-microbe competition shapes nutrient turnover in the rhizosphere

We observed consistent patterns in the abundance of microorganisms in the rhizosphere at both sites, irrespective of the fertilization treatment (Fig. S4). Bacteria carrying the low-affinity phosphate transporter gene (*pitA*) displayed similar abundance across soil compartments, whereas bacteria harboring the *pstS* gene were more abundant in the rhizosphere, indicating an intensified competition for phosphate at the root-soil interface. Studies showed that plants actively absorb nutrients from the rhizosphere, leading to localized depletion zones (Kreuzeder et al., 2018; York et al., 2016), which is intensified by an increase in microbial growth on plant derived C (Bulgarelli et al., 2013). Our findings of higher DC in the rhizosphere at both sites (Table S5) confirm the influence of root exudates in creating a nutrient rich micro environment favorable for microbial activity.

These results align with Lidbury et al. (2022), who observed that even in soils saturated with inorganic fertilizers, *Pseudomonas* species in the rhizosphere experienced P-limited conditions, as evidenced by increased PstS. A similar pattern was observed in our study for bacteria harboring the *phoD* gene, which were more abundant in the rhizosphere, consistent with other studies linking *phoD* abundance to active P acquisition in the rhizosphere (Khan et al., 2023; Li et al., 2023). In contrast, *gcd* haboring bacteria were similarly abundant in both soil compartments (Fig. S4).

No differences in abundance were observed for bacteria encoding the *nxrA* gene and denitrifiers carrying the *nirS* gene between bulk soil and rhizosphere. However, bacteria harboring the *nirK* gene showed reduced abundance in the rhizosphere, suggesting that *nirK*-type denitrifies, which are known for their broader substrate utilization, may be less dependent on root-derived substrates in this zone (Hou et al., 2018). As for *nirK* denitrifiers, AOB also showed no significant differences between soil compartments, indicating they may not specifically rely on the rhizosphere for their abundance (Wattenburger et al., 2020).

Interestingly, AOA did not exhibit higher abundance in the rhizosphere and were even less abundant at the KB site, where NH_4^+ levels were lower. A higher abundance of AMF in the KB rhizosphere may have contributed to this trend, as AMF are known to compete with NH_4^+ oxidizers (Bollmann et al., 2002; Juliette et al., 1993). In contrast, bacteria carrying the *nosZ* gene, associated with denitrification, did not show an increased abundance in the rhizosphere at KB. This observation aligns with Nie et al. (2014), who found that *nosZ* carrying bacteria tend to be less abundant in rhizosphere soil than bulk soil, likely due to distinct ecological niches and substrate availability.

Differences in microbial nutrient turnover between bulk soil and rhizosphere are well documented (Ai et al., 2012; Hess and Austin, 2017). However, recent studies have increasingly focused on fertilizer impacts on bulk soil bacterial communities (Dai et al., 2018; Grafe et al., 2021). Assessing fertilizer treatments across all soil compartments is crucial, as fertilization regulates nutrient competition between soil microbes and plants.

5. Conclusions

This study tested different P fertilization treatments in two on-farm trials. Our findings show that adaptations to P fertilizer types are site-specific. Higher AMF abundance and ALP activity stabilized plant nutrient ratios at the silty loam site, while higher available P at the second site supported nutrient balance through P-solubilizing bacteria. These findings underline the role of soil- and site-specific mechanisms in nutrient cycling. Additionally, it demonstrates a distinct untargeted effect of novel P fertilizers on N turnover processes across both sites, emphasizing the close link and cross-reaction of different nutrient cycles. Future research should expand on these findings by conducting additional on-farm studies that reflect the complexity and natural variability of real agricultural systems, which cannot be fully replicated in controlled trials. Furthermore, while this research highlighted the site-specific adaptation to P fertilizers, the study was conducted at only two sites, limiting broader generalizations, but allowing us to exclude further variables like differences in temperature and precipitation. Expanding research to include farms with diverse soils, climates, and management systems will be critical for understanding the resilience and adaptability of nutrient dynamics under varying conditions and for developing sustainable, site-specific nutrient management practices.

CRediT authorship contribution statement

Schulz Stefanie: Writing – review & editing, Supervision, Conceptualization. **Baum Christel:** Writing – review & editing, Conceptualization. **Leinweber Peter:** Writing – review & editing, Funding acquisition. **Panten Kerstin:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Schloter Michael:** Writing – review & editing, Funding acquisition, Conceptualization. **Thaqi Stefanie Katharina:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Siani Roberto:** Writing – review & editing, Methodology, Formal analysis. **Chiba Akane:** Writing – review & editing, Resources, Investigation. **Vitow Nora:** Writing – review & editing, Resources, Investigation.

Declaration of Competing 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109565.

Data Availability

Data will be made available on request.

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