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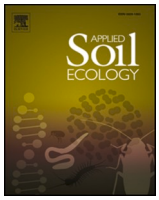

Research paper

# Unraveling the impact of protein hydrolysates on rhizosphere microbial communities: Source matters

Ohana Y.A. Costa [a], Jingjing Chang [a], Ji Li [a], Willem van Lith [b], Eiko E. Kuramae [a,c,*]

[a] Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), 6708PB Wageningen, the Netherlands
[b] Innovative Seed Technologies BV, Hengstdalseweg 44, 6523 EM Nijmegen, the Netherlands
[c] Ecology and Biodiversity Group, Department of Biology, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584, CH, Utrecht, the Netherlands

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ABSTRACT

Protein hydrolysates (PHs), derived from enzymatic or chemical protein hydrolysis, are recognized as effective biostimulants for sustainable and environmentally safe crop production. Extensive research has highlighted their benefits and demonstrated their capacity to enhance crop growth and yield under various abiotic stresses, making them increasingly popular in agriculture. To fully unlock the potential of PHs, more research is needed to elucidate their mechanisms of action. This involves understanding plant preferences for different PH sources as well as their impact on rhizosphere microbial communities. This study explored how PHs from plant and animal sources affect plant growth and rhizosphere microbiota across five different plant species. We found variations in plant responses to different PHs, indicating differing plant preferences for nitrogen sources and protein uptake mechanisms among species. There was an increase in beneficial microbial taxa in response to PH application, including *Pseudomonas*, *Paraburkholderia*, and *Mortierella*. Functional analysis also indicated variations in chemoheterotrophy, nitrate respiration and reduction, based on crop species. In conclusion, this research shows the potential of PHs as biostimulants for diverse crops. Their effectiveness is dependent on various factors, including source, production process and plant species, having a positive impact on both plant growth and rhizosphere microbial communities.

## 1. Introduction

Protein hydrolysates (PHs), which are derived from the enzymatic or chemical hydrolysis of proteins, are recognized as biostimulants that promote environmentally safe and sustainable crop production systems (Colla et al., 2017). Extensive research has highlighted the diverse benefits of PHs on crop physiology, including increased carbon and nitrogen metabolism, enhanced secondary metabolism, up-regulation of genes related to nitrate uptake, and stimulation of enzymatic activities for iron uptake from roots (Celletti et al., 2020; Ertani et al., 2009; Nardi et al., 2016; Schiavon et al., 2008). Furthermore, PHs have been shown to positively impact crop growth and yield under various abiotic stresses, even at low application rates, making them a popular subject of study and use in agriculture (Colla et al., 2015; Francesca et al., 2022; Sorrentino et al., 2021).

Currently, most PHs are derived from plants, with several experimental studies demonstrating their effectiveness in stimulating shoot and root biomass, resulting in increased productivity of various crops (Colla et al., 2014; Schiavon et al., 2008). In addition to plant-derived PHs, there is also growing interest in animal-derived PHs, such as gelatin. Gelatin, an animal-derived protein, has been used as capsules placed near seeds and has been shown to act as a biostimulant on different crops (i.e. cucumber, arugula, broccoli, tomato, pepper) (Touchette and Cox, 2022; Wilson et al., 2018, 2015). Furthermore, our previous work also proved that gelatin is a good candidate for enrichment of beneficial microbes such as the genera *Trichoderma*, *Burkholderia*, *Rhodanobacter*, *Pseudomonas*, etc. (Costa et al., 2023) in different soils and substrates. Therefore, it shows that PHs can also improve soil microbial activities, which might be indirectly enhancing plants biomass and nutrient uptake through nutrient release and hormone production (Colla et al., 2017).

However, to fully realize the potential of PHs, further studies are needed to elucidate their action mechanisms, which include understanding plant preferences for different sources of PHs, whether the structure and composition of rhizosphere microbial communities are influenced by different sources of PHs, and identifying unique core

* Corresponding author at: Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), 6708PB Wageningen, the Netherlands.
*E-mail address:* e.kuramae@nioo.knaw.nl (E.E. Kuramae).

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microbial groups influenced by different sources of PHs. We hypothesized that PHs from different sources recruit unique core microbial communities, resulting in varying effects on promoting plant growth. Therefore, to further elucidate the effects of PHs from plant and animal sources on plant growth and rhizosphere microbiota, this study applied these two protein hydrolysates to five different plant varieties and conducted biomass measurements after a two-week period. Additionally, bacterial and fungal communities in the rhizosphere were analyzed using amplicon sequencing targeting the 16S rRNA partial gene for bacteria and the internal transcribed spacer (ITS) region for fungi.

## 2. Methods

### 2.1. Experimental plant varieties

Five different plants varieties, namely courgette (*Curcubita pepo* L.), melon (*Cucumis melo* L.), pumpkin (*Curcubita maxima* L.), tomato (*Solanum lycopersicum* L.), and snack paprika "Arwen" (*Capsicum annuum*), were used to test the effects of various protein hydrolysates on plant growth promotion and microorganisms. Seeds were surface sterilized with a solution containing 1.5 % bleach for 20 min and then rinsed 5 times with sterilized demi water. Seeds were sown in plastic pots filled with 21 g dry weight of seedling soil (80 % peat products, fine particles, 40 % German/Irish peat, 40 % Swedish peat, 20 % Perlite 2 fine) (LENSLI®, Bleiswijk, Netherlands).

### 2.2. Treatments

To compare the effects of gelatin capsules (CAP) (#3 hard-gel animal-based gelatin capsules, Torpac Europe BV, Herleen, The Netherlands) and pea protein (PEA) hydrolysates, a bulk control (CTRL) with no additives was also included, along with the use of urea (a mineral fertilizer from Sigma-Aldrich, Missouri, USA; UREA) as a positive control. The dosage for CAP was 2 capsules per pot (Wilson et al., 2018), while PEA was 109 mg per pot and UREA was 30 mg per pot, therefore each treatment containing 14.2 mg of nitrogen. For each treatment there were 15 pots replicates. The plants were grown in a growth chamber that was controlled at a temperature of 25 °C/21 °C, with a 16/8-h photoperiod and 75 % relative humidity, at the Netherlands Institute of Ecology (NIOO-KNAW). Plants were watered as needed every other day throughout the experiment, keeping 40 % of substrate water holding capacity (2.02 g of water/g substrate).

### 2.3. Plant growth analysis and data processing

The plant biomass was evaluated upon second true leaf stage or when seedlings were ready for transfer, in case of tomato and paprika (tomato – 16 days after germination, paprika – 23 days after germination, courgette and pumpkin – 9 days after germination, melon - 16 days after germination), including measurements of fresh and dry shoot and root weights, as well as leaf area. Specifically, we grouped fresh weight data for five shoots and roots. In the case of tomato and paprika plants, we scanned the entire plant area. For melon, courgette, and pumpkin, we scanned the cotyledon and first leaf area ($cm^2$). The area of plants leaves was scanned and measured with "WinFOLIA Pro 2016" software (Regent Instruments, Inc., Canada). Additionally, we obtained five rhizosphere soil samples for each plant, for each treatment, by carefully uprooting them from the pots and brushing the soil attached to the roots.

Total soil DNA was extracted from 0.25 g of soil using the MoBio PowerSoil DNA Isolation Kit (MO BIO, Solana Beach, CA, USA) according to the manufacturer's instructions. DNA concentration was determined using NanoDrop spectrophotometer (Thermo Scientific, USA) and DNA integrity was assessed by 1 % (*w/v*) agarose gel electrophoresis. The DNA extracted from the soil was used for amplification and sequencing of the 16S rRNA partial gene and ITS1 region. The forward primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3′) and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3′) were used to target the V3-V4 region of the 16S rRNA gene for bacteria. For fungi, the ITS1 region was targeted using ITS1F (5′- CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) primers. Dual-index Illumina sequencing adapters were attached to the amplicons. After library quantification, normalization and pooling, MiSeq V3 reagent kits were used to prepare the samples for MiSeq sequencing. The samples were sequenced on the Illumina MiSeq System at Genome Québec, Montréal, Canada. Raw DNA sequences were processed with the Cutadapt v2020.11.1 (Martin, 2011). DADA2, as implemented in qiime2 v2019–08 (Bolyen et al., 2019; Callahan et al., 2016), was used to process forward and reverse reads for identification of amplicon variant sequences (AVSs). Quality trimming, denoising, merging, and chimera detection were performed using the qiime2 v2020.11.1 plugin "qiime dada2 denoise-paired" with default settings, except for "–p-trunc-len-f" and "–p-trunc-len-r" which were set at 200 and 180 nucleotides, respectively, for ITS regions, and 200 and 190 for 16S rRNA gene. Taxonomic lineage of the representative sequences of resulting ASVs was classified using the classify-sklearn plugin (Pedregosa et al., 2011) and SILVA database (v.138) (Quast et al., 2013) for bacterial profiles, and Unite (v. 8.2) (Kõljalg et al., 2013) for fungal profiles. ASV tables were then converted into tab-separated values (tsv) format and exported using the BIOM package (McMurdie and Paulson, 2020). Potential functions in the bacterial community were determined using the FAPROTAX database (Sansupa et al., 2021).

### 2.4. Statistical analyses

The plants biomass data statistical processing was performed using the Shapiro–Wilk test, followed by statistical processing using one-way analysis of variance (ANOVA) and a Tukey HSD post hoc test at the $P < 0.05$ level. GraphPad Prism (v.9.0.0) software was used to visualize the graphing results. Principal coordinates analysis (PCoA) matrices were used to visualize the microbial community structure of samples, using the phyloseq package and the variance partitioning was calculated using the permutational multivariate analysis of variance PERMANOVA ($P < 0.05$) model based on Bray-Curtis using the adonis function in the vegan package (Oksanen et al., 2018). Generalized Joint Attribute Modeling (GJAM) package (Clark et al., 2017) was used to estimate the effects of different protein hydrolysates on microbial community composition and bacterial potential functions. We evaluated the relevance of different microbes for the soil community in terms of centered log-ratio (CLR) transformed abundance. CLR transformation demonstrates the relevance of different microbial groups as a proportion of the sample's average. This transformation was applied to classify the soil microbes as originally highly or lowly abundant, based on log-fold differences in comparison to the average, which corresponds to the zero value for CLR transformed data. A Hierarchical Clustering on Principal Components (HCPC) analysis was carried out to identify the main clusters affected by the different protein hydrolysates using the FactoMineR package (Lê et al., 2008). The GJAM and HCPC analysis were performed in the R (v.4.1.3) platform (R Core Team, 2015).

## 3. Results

### 3.1. Plant biomass

The plant biomass measurements indicated that the application of CAP and PEA protein treatments resulted in a significant increase in the fresh weight and dry weight of courgette roots, shoots, and the area of the first leaf, when compared to the control (CTRL) (Fig. 1A). PEA was determined to be more effective than other treatments in promoting the aboveground biomass of melon, tomato, and paprika, as evidenced by a significant increase in shoot fresh weight and leaf areas (Fig. 1B, D, E). However, the addition of PHs and UREA did not significantly affect the shoot and root weight of pumpkin (Fig. 1C). Overall, courgette was

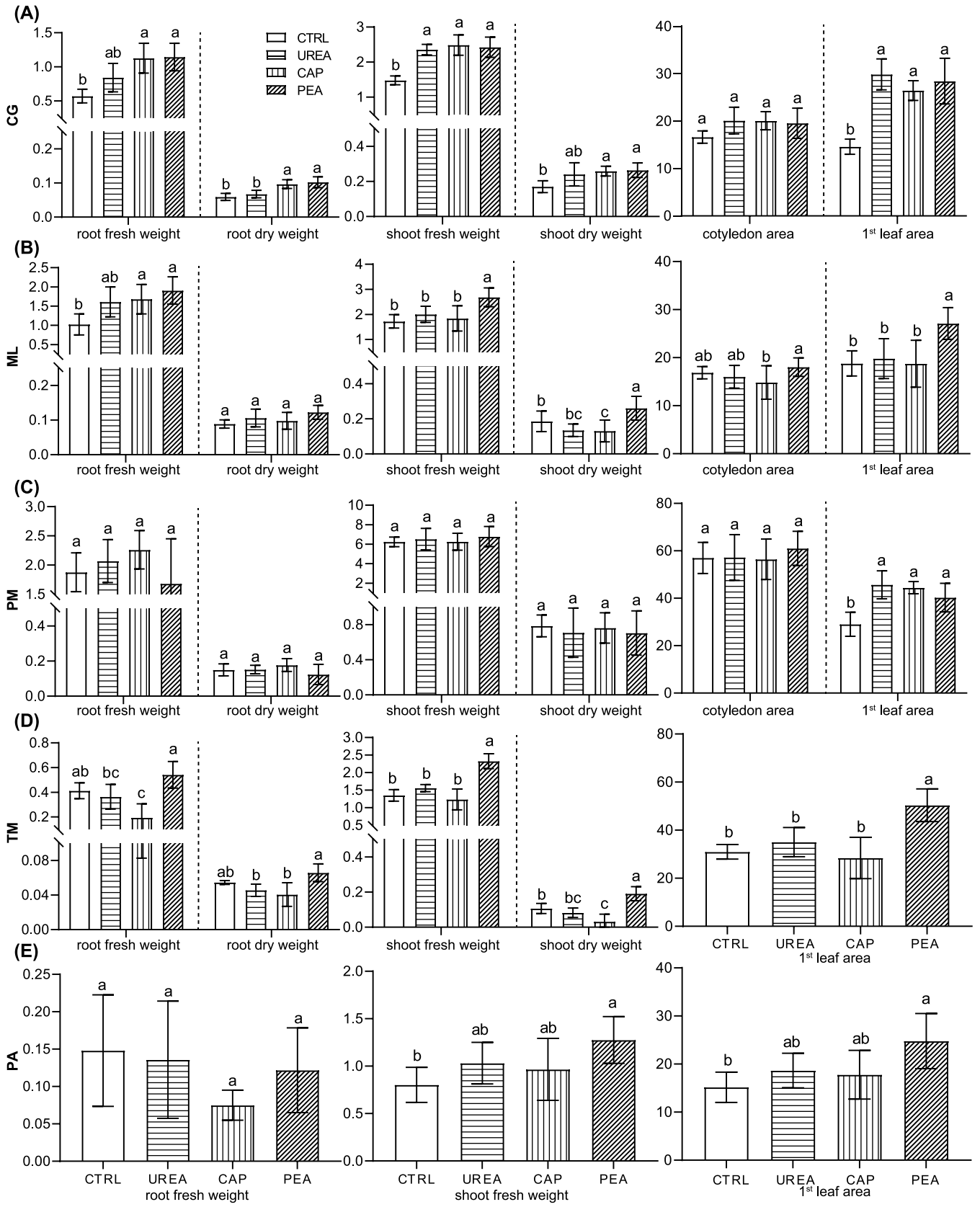


**Fig. 1.** Plant parameters measured at all timepoints. Root biomass, shoot biomass and1st leaf area ($cm^2$). A) CG (courgette), B) ML (melon), C) PM (pumpkin), D) TM (tomato), E) PA (paprika). CTRL (control with no treatment), urea (mineral fertilizer), two gelatin capsules (CAP), pea protein (PEA). Data analyzed with one-way Analysis of Variance (ANOVA, $\alpha = 0.05$) and a Tukey HSD post hoc test at the $P < 0.05$ level. Analyses. Different letters indicate significant difference among treatments.

found to be the most responsive to the treatments with PHs and UREA. PEA showed high potential in promoting the growth of courgette, melon, tomato, and paprika.

### 3.2. Microbial communities

The analysis of the 16S rRNA amplicon sequences revealed the presence of 2802 bacterial amplicon sequence variants (ASVs) across 26 phyla in all samples. The predominant phyla identified were *Pseudomonadota* (47.99 % ~ 18.10 %), *Actinobacteriota* (42.10 % ~ 14.40 %), and *Planctomycetota* (16.94 % ~ 6.31 %) (Fig. S1A). Additionally, a total of 1908 fungal ASVs were identified across five phyla in all samples, including *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota* and *Rozellomycota* (Fig. S1B).

Principal coordinates analysis (PCoA) was performed using Bray-Curtis differences based on ASV abundance to assess the impact of PHs across various plants (Fig. 2). Our analysis revealed that the CAP, PEA, UREA treatments can significantly change the bacterial communities across all studied plant species, although the variation also influenced by the plant species ($R^2 = 0.60$, $P \leq 0.001$; Fig. 2A). This finding is further supported by the PERMANOVA results, which indicate that PEA had the greatest impact on soil bacterial communities in courgette, tomato, and paprika, while CAP had the greatest impact in melon and pumpkin (Table S1).

In addition, CAP and PEA had a greater effect on bacterial community structure than UREA in all studied plant rhizosphere soils except pumpkin. In contrast, the fungal community structure was more affected by the plant species than the treatments (Treatment: $R^2 = 0.09$, $P \leq 0.001$; Plant: $R^2 = 0.18$, $P \leq 0.001$; Fig. 2B). Moreover, the sample distribution also clearly indicates that PEA was located far away from all other treatments in terms of bacteria, and paprika was distant from all other plants in terms of fungi (Fig. 2).

Therefore, it can be confirmed that PEA have a greater influence on bacterial communities compared to fungi. The GJAM analysis indicates that the PHs of CAP and PEA can have a significant impact on the microbial community composition across various plants (Figs. 3, 4). In all studied plants except pumpkin, the soil microbial community composition was most influenced by PEA treatment compared with UREA and CAP (Fig. S2). Specifically, a significant impact of PEA was observed on a range of 21 to 37 bacterial genera groups and 24 to 32 fungal genera groups across all the plants (Fig. S2). PEA positively impacted 16.22 % to 25.68 % of bacterial genera, and negatively impacted 10.81 % to 25.68 % of bacterial genera. In terms of fungi, PEA positively affected around 13.70 % to 16.44 % of fungal genera, while negatively impacting 19.18 % to 27.40 % of fungal genera (Table S2). In all studied plants, the bacterial genera that were predominantly positively affected by PEA included *Pseudomonas*, *Labilithrix*, *Sphingomonas*, *Tumebacillus*, and *Devosia* (Fig. 3B). Notably, *Pseudomonas*, *Labilithrix*, and *Tumebacillus* exhibited the most significant increase in response to PEA (Fig. 3B). Among the genera groups significantly increased by CAP,*Labilithrix*, *Rhodanobacter*, *Dyella*, *Bordetella*, *Paraburkholderia* were among the top 5 genera that showed the most positive impact due to CAP (Fig. 3A). Conversely, UREA only positively significantly increased the relevance of genus *Massilia* for all the plants (Fig. 3C). Among the genera the most frequently negatively impacted by the treatments were unclassified bacteria, *Portiococcus*, *Tumebacillus*, *Actinospica* and *Methylobacillus* (Fig. 3).

Similarly to the bacterial community, the analysis of the bacterial functions with FAPROTAX demonstrated that different functions were affected by the treatments depending on the plant. CAP influenced aerobic chemoheterotrophy positively in pumpkin, tomato and courgette, nitrate respiration in melon and ureolysis in paprika (Fig. S3A). PEA did not affect positively any function in paprika, but increased the relevance of nitrogen and nitrate respiration in pumpkin and courgette, as well as chemoheterotrophy in tomato, methanol oxidation in melon and courgette, as well as dark thiosulfate oxidation in melon (Fig. S3B). Urea increased the relevance of dark oxidation of sulfur compounds in most of the plants, except for courgette, in which it was affected negatively (Fig. S3C).

In most of the plants, except for paprika, PEA increased the relevance of genera such as *Mortierella*, *Leucoprinus*, *Fusarium, Cutaneotrichosporon* and *Giberella,* while for paprika, *Mortierella* was not positively impacted. (Fig. 4B). For the other treatments, no genus had their relevance increased for all the plants in general. Treatment with CAP impacted positively several different genera among the studied plants such as *Cutaneotrichosporon*, *Slooffia*, *Leucoprinus*, *Conlarium* and *Acrodontium*, (Fig. 4A). Urea also increased the relevance of different genera for each plant, such as *Giberella*, *Fusarium*, *Byssochlamys* and *Leucoprinus*

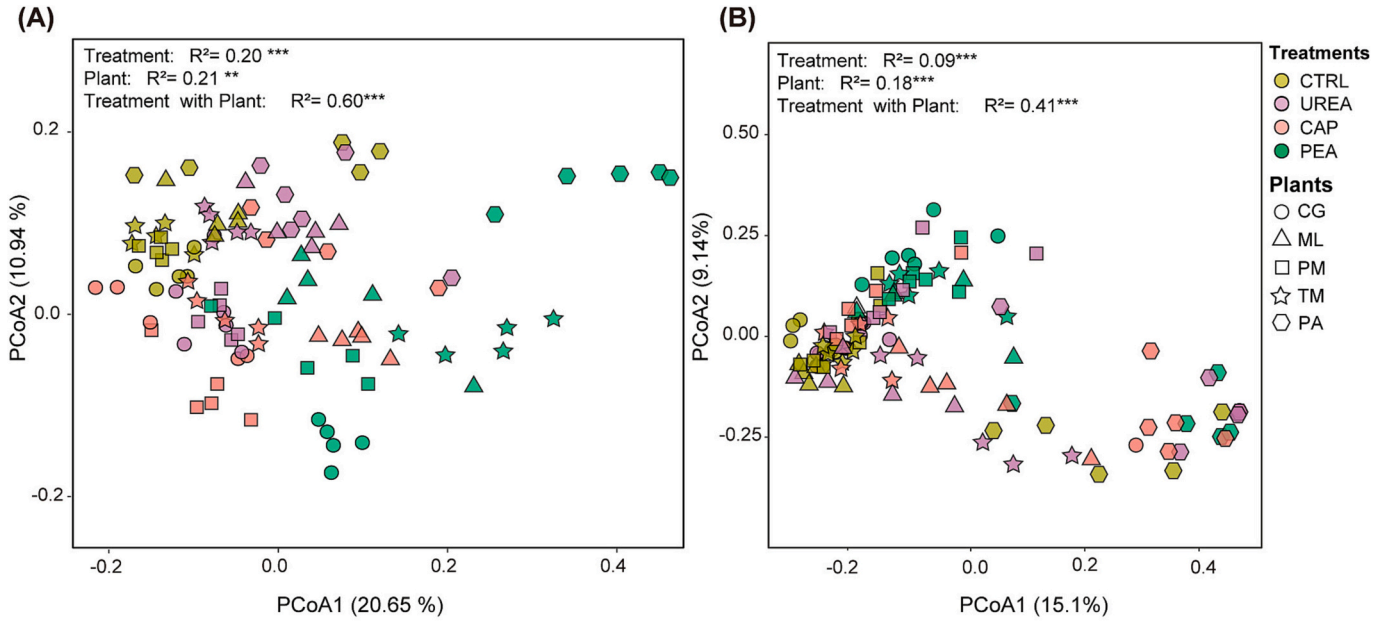


**Fig. 2.** Principal coordinate analysis (PCoA) based on the Bray-Curtis distance. A) bacterial and B) fungal ASVs. Variance partitioning was calculated using the permutational multivariate analysis of variance PERMANOVA ($P < 0.05$). CG (courgette), ML (melon), PM (pumpkin), TM (tomato), PA (paprika). CTRL (control with no treatment), urea (mineral fertilizer), two gelatin capsules (CAP), pea protein (PEA).

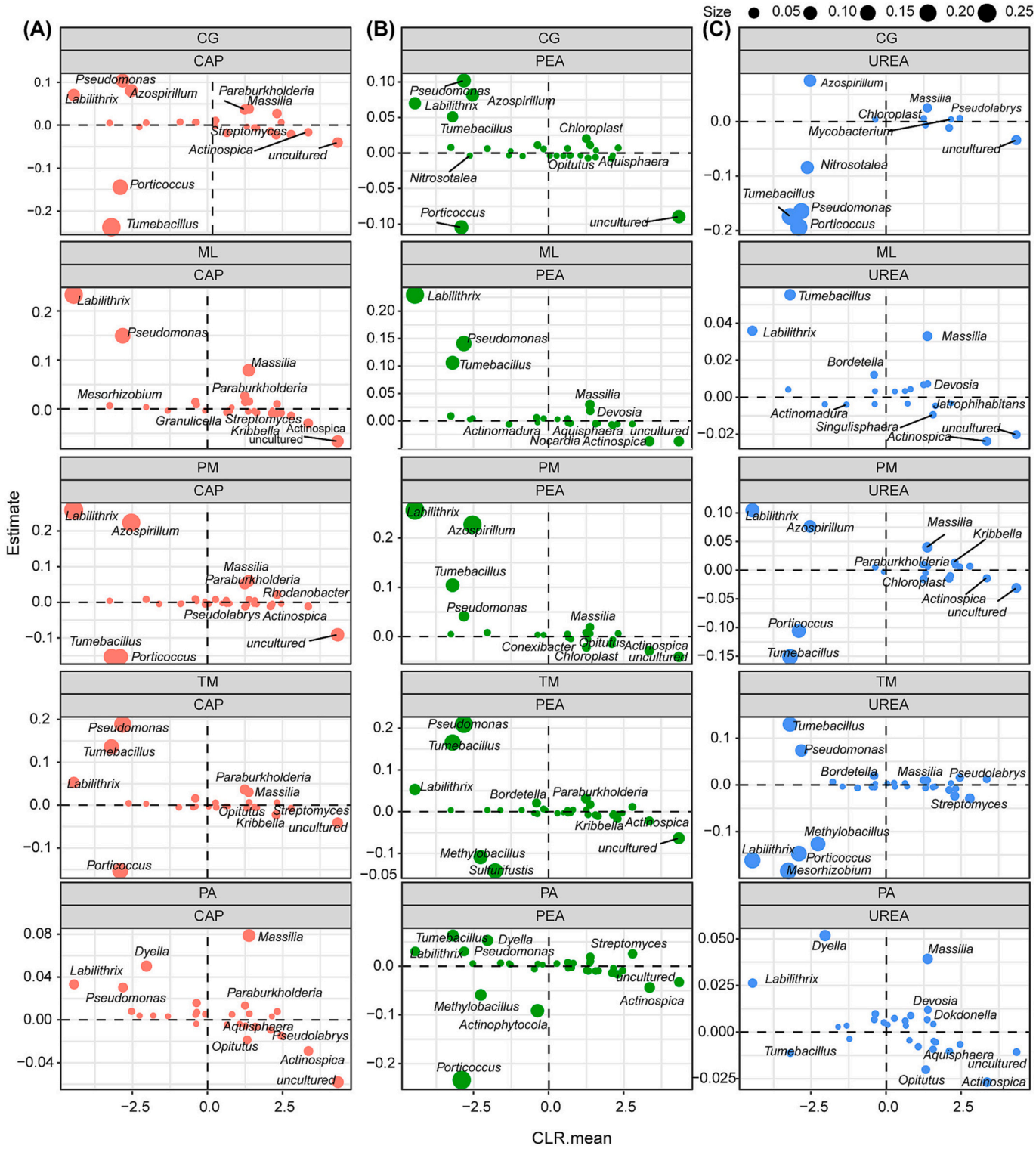


**Fig. 3.** Relationship between the regression coefficient and the abundance (CLR-transformed). Identification of the 5 most negative and most positive shifts from bacterial taxa relatively to the control treatments. A) two gelatin capsules (CAP), B) pea protein (PEA) C) urea (mineral fertilizer), CG (courgette), ML (melon), PM (pumpkin), TM (tomato), PA (paprika).

(Fig. 4C). In addition, several genera had their relevance decreased by the treatments, varying for each plant. *Meyerozyma*, *Pezoloma*, *Apiotrichum*, *Oidiodendon* and *Metapochonia* were the top genera among the more frequently negatively affected by the treatments in all the plants.

## 4. Discussion

The application of PHs in agriculture is a promising and more sustainable tool for improving crop productivity, as an alternative to mineral fertilizers. Nonetheless, the impact of protein hydrolysates in the microbial communities of crops has not yet been sufficiently

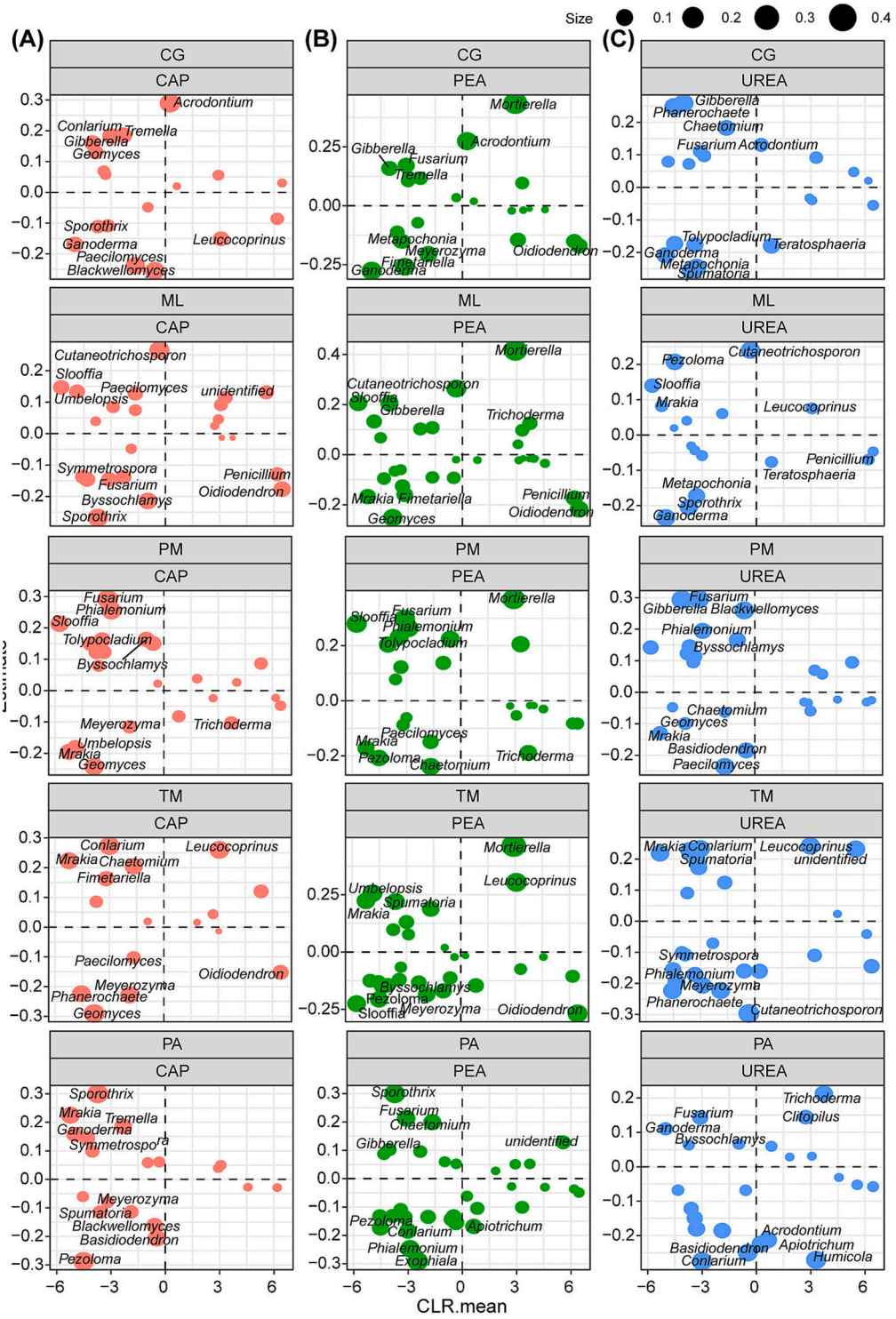


**Fig. 4.** Relationship between the regression coefficient and the abundance (CLR-transformed). Identification of the 5 most negative and most positive shifts from fungal taxa relatively to the control treatments. A) two gelatin capsules (CAP), B) pea protein (PEA) C) urea (mineral fertilizer), CG (courgette), ML (melon), PM (pumpkin), TM (tomato), PA (paprika).

investigated. In this study, we examined the impact of two protein hydrolysates (animal – gelatin and plant-based – pea protein) in the plant growth and rhizosphere-associated microbial community of five crops: courgette, melon, pumpkin, tomato and paprika. As expected, the response of the plants to the PHs varied. Our results showed that, while pumpkin was not affected by the addition of nitrogen sources, the growth of courgette was enhanced by both pea protein and gelatin, and pea protein also promoted growth of melon, tomato and paprika. These results can be associated to the plant preferences for different nitrogen sources, for instance, organic versus inorganic nitrogen (Wilson et al., 2018), as well as different mechanisms for protein uptake, and their availability, which may vary depending on the plant species.

The positive effect of PHs as biostimulants has been observed for several crops; nonetheless, several factors might influence the effect of PHs, such as formulation, dose, time and type of application, plant species, plant genotype, developmental stage and environmental

conditions (Caruso et al., 2020; Francesca et al., 2022). For instance, gelatin capsules enhanced shoot dry weight of cucumber, pepper, broccoli, tomato, arugula and field corn in up to 244 %, with the magnitude of the effect depending on the species (Wilson et al., 2018). Chicken feather-derived protein hydrolysate impacted positively plant performance and yield in mung beans, enhancing germination frequency, root weight, protein content in plants and also soil fertility (Kaur et al., 2021). Feather degradation products had a similar positive effect for banana, increasing the phenolic and flavonoid compound contents in fruits and in the plant (Gurav and Jadhav, 2013). The improvement of leaf yield, number of leaves per rosette, lipophilic and hydrophilic antioxidant activity as well as ascorbic acid and chlorophyll *b* content were observed for wall rocket after the application of a legume-derived material (Caruso et al., 2020). Plant-derived commercial protein hydrolysate 'Trainer" promoted plant growth and enhanced nitrogen contend from maize and lettuce plants, using full strength or diluted solutions for foliar application (Colla et al., 2013).

In addition to the plant species effect, differences in the action of PH might be due to their chemical characteristics, which will vary depending on protein source (animal, plant-based) and production process (chemical/enzymatic hydrolysis) (Colla et al., 2017). Acid hydrolysis is very aggressive, resulting in a large amount of free amino acids, while also destroying amino acids such as tryptophan and cysteine and inactivating others (Colla et al., 2017, 2015). Enzymatic hydrolysis, however, produces hydrolysates containing higher proportions of biologically active peptides (Colla et al., 2017). Peptides are considered to be more potent antioxidants than free amino acids, due to the higher peptide stability. Furthermore, their action is dependent on the amino acid composition and sequence (Abuine et al., 2019; Korkmaz and Tokur, 2022). The direct effect of PHs is usually attributed to low molecular size particles, that are readily available to plant leaves and roots, act metabolic regulators. PHs can also contain endogenous hormones, phenolics and triacontanol, stimulating pathway that induce, for instance, root development (Caruso et al., 2020; Ertani et al., 2014).

A direct effect of PHs on plant metabolism will directly affect the microbial community associated to the crops, since plants can alter the soil microbiota by secreting root exudates containing bioactive molecules (Colla et al., 2017). The absorption of a variety of nutrients and the action of hormone-like molecules can induce the modification of root exudates and metabolites (Colla et al., 2015; Tekaya et al., 2021), promoting the modification of the rhizosphere microbial community. Root exudates are usually composed of diverse metabolites, such as sugars, amino acids and carboxylic acids, as well as other molecules (Hartmann et al., 2009). These compounds can be used as nitrogen and carbon sources for microbes, but also act as signaling molecules, attracting and stimulating or inhibiting and repelling microbes (Baetz and Martinoia, 2014). In addition, the composition of root exudates depends on host-genetic control, varying substantially among plant species, impacting the recruitment of the rhizospheric microbial community (Bulgarelli et al., 2013; Hu et al., 2018). For instance, (Eilers et al., 2010) observed that the addition of low molecular weight carbon substrates to different soils induced the preferential increase in relative abundance of specific bacterial taxa, with a significant response of β-Proteobacteria to citric acid. Accordingly, our study showed that, independently of the PH applied to the crops, the effect of the crops on the structure of the microbial community was stronger, even if the plant substrate used was the same. For example, the fungal genus *Mortierella* is highly abundant and positively impacted in most of the crops, except paprika, demonstrating the influence of the plant species in the microbes recruited. In addition, for both bacterial and fungal communities, there was no core of taxa that were positively or negatively impacted either for all crops or all treatments. Such results reinforce the need of more studies concerning the impact of biostimulants in the rhizospheric microbial communities of different crops, since the effect can be quite variable.

Interestingly, the positive effect of PHs may also be associated to the beneficial microbes recruited by the plant (Philippot et al., 2013). Rhizospheric microbes can be directly involved in the plant performance, through nutrient acquisition, pathogen control, nutrient cycling and decomposition processes (Colla et al., 2017; Van Der Heijden et al., 2006). Therefore, biostimulants can enhance plant performance by acting indirectly through the enrichment of plant beneficial microbes (Colla et al., 2017). The plant microbiome can enhance plant growth through a variety of pathways, for instance production of phytohormones (auxin, cytokinin, gibberellin), nitrogen fixation, phosphorus solubilization, siderophore production and production 1-aminocyclopropane- 1-carboxylate (ACC) deaminase, which reduces plant stress (Babalola et al., 2020; Nadeem et al., 2015).

In this context, the investigation of the microbial communities impacted by PHs and other non-microbial biostimulants lead to potential beneficial candidates, which can be isolated in further studies and either applied alone or in combination with biostimulants.

Our results demonstrated that the application of PHs increased the relevance of several taxa containing beneficial traits, such as *Pseudomonas*, *Tumebacillus*, *Paraburkholderia*, *Rhodanobacter*, *Mortierella*, *Fusarium*, *Leucoprinus*, and others. For instance, it was observed that some strains of *Pseudomonas*, can produce the enzyme 1-aminocyclopropane- 1-carboxylate (ACC) deaminase, related to indirectly promoting plant growth by decreasing plant stress (Babalola et al., 2020; Rascovan et al., 2016). In addition, members of the genus *Pseudomonas* are able to secrete enzymes, such as proteases, lipases, phosphatases and chitinases (Tielen et al., 2010). Another enzyme producer is *Tumebacillus,* which genus contains soil isolates capable of producing enzymes such as amylases (Wang et al., 2013). *Labilithrix* genus has been isolated from forest soil and is considered of particular interest due the production of secondary metabolites with antiviral and antimicrobial activities (Mulwa et al., 2018). *Paraburkholderia* genus is a promising group of microbes, harboring many species capable of plant-growth promotion properties, such as nitrogen fixation (Vio et al., 2020). The genus *Rhodanobacter* has been related to the degradation of aromatic compounds (Song et al., 2016) and forest litter (Verastegui et al., 2014), producing extracellular proteases and lipases (Lee et al., 2014). *Mortierella* and *Leucoprinus* are saprotrophs, observed in bulk soil, rhizosphere and plant tissue, with potential to improve access to nutrients and produce phytohormones, promoting also protection against pathogens (Ozimek and Hanaka, 2020). Saprotrophs are in general good biopolymer decomposers and enzyme producers, hydrolyzing and mobilizing nutrients from organic matter (Baldrian et al., 2011; Phillips et al., 2014). In addition, they can enhance litter decay and do further nutrient mineralization, maintaining and enhancing soil fertility (Hellequin et al., 2020). Members of *Fusarium/Giberella* are highly abundant in soil microbial communities (Crous et al., 2021), producing a wide range of extracellular enzymes, such as glycosyl hydrolases and proteases (Kwon et al., 2007; Perincherry et al., 2020). *Cutaneotrichosporon* genus contains oleaginous yeasts capable of degrading several carbon sources, such as glucose, xylose and glycerol, as well as complex biomass waste (Awad et al., 2019). *Tremella* is a plant-associated genus capable of assimilating several carbon sources (Khunnamwong et al., 2019).

In addition to the microbial taxa, we analyzed the potential bacterial functions using FAPROTAX, which results also varied according to the crop species. FAPROTAX is a predictive tool, which, despite its limited database, it is an interesting alternative to explore the potential of 16S rRNA datasets without associated metagenomics data. Pea protein impacted positively the relevance of functions related to the nitrogen cycle in pumpkin and courgette, particularly nitrogen and nitrate respiration, as well as nitrate reduction, consistent with the addition of organic nitrogen sources to the substrate. Gelatin and pea protein increased the relevance of chemoheterotrophy, which is related to the carbon cycle. Nonetheless, the majority of other functions are broad and related to several different taxa (Jung et al., 2021). Increased nutrient availability, especially of N sources, is fundamental for plant growth, affecting several functions, such as time and rate of seed germination, leaf expansion and function, as well as root and shoot architecture

(Andrews et al., 2013).

## 5. Conclusions

Employing pea protein hydrolysates (PHs) across diverse crops has emerged as a successful biostimulant strategy, yielding enhanced plant growth, notably in terms of fresh shoot weight and initial leaf area. The results had variable degrees of success, showing that the application of other materials should be tested in further studies. The introduction of all PHs into the rhizosphere increased the significance of beneficial taxa associated with plant growth, such as *Pseudomonas*, *Paraburkholderia* and *Mortierella*. This enhancement is attributed to the synthesis of diverse bioactive compounds and the protection against plant pathogens. While a deeper comprehension of the specific mechanisms behind plant growth for each PH is needed for refined control, it is evident that microbial communities play a pivotal role in both plant growth and the action of PHs. The adoption of protein-based biostimulants has demonstrated effectiveness as an alternative to mineral fertilizers, promoting favorable impacts on both plants and the microbial community. Furthermore, this approach holds potential for further exploration in uncovering novel microbial biostimulants that can be employed independently or synergistically to fully exploit the efficacy of PHs.

## Statements and declarations

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## CRediT authorship contribution statement

**Ohana Y.A. Costa:** Conceptualization, Investigation, Methodology, Writing – original draft. **Jingjing Chang:** Data curation, Methodology. **Ji Li:** Formal analysis, Methodology. **Willem van Lith:** Conceptualization, Resources, Supervision. **Eiko E. Kuramae:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

## 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.

## Data availability

The datasets supporting the conclusions of this article are available in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) under the accession number PRJEB66732.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105307.

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