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REVIEW ARTICLE

INCREASE IN THE ACTIVITY OF MICROBIAL AGENTS OF AGRICULTURAL INTEREST USING A BIOSTIMULANT

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ABSTRACT

Global agriculture is rapidly adopting bioinputs for the sustainable management of crops. The use of symbiotic fungi and bacteria marks a significant technological advance towards cleaner, more efficient, and regenerative farming practices. Stimulating the activity of beneficial microorganisms in the soil is crucial for achieving high productivity while minimizing environmental impact. In this study, microbial agents commonly used in global agriculture were evaluated for their response to the biostimulant product Penergetic "p". In vitro tests were conducted using both liquid and semi-solid fermentation of biological agents, with and without the biostimulant in the culture medium. Microbial growth and development were assessed based on growth diameter and spore count. The results showed that Penergetic "p" effectively stimulated the growth and development of all evaluated microbial agents, with average increases of over 35% for fungi and 130% for bacteria. This biostimulant has proven to be an efficient tool for enhancing the activity of microbial agents in agriculture.

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INTRODUCTION

The importance of maintaining and stimulating the soil microbiome in agriculture is a highly relevant topic, given its substantial impact on both agricultural productivity and environmental sustainability (Cao *et al*., 2021; Feng *et al*., 2022; Zhang *et al.,* 2024). Soil microorganisms play crucial roles in nutrient cycling, organic waste decomposition, nutrient solubilization, disease suppression, the maintenance of microbial communities in the rhizosphere, and the promotion of plant growth (Ma *et al*., 2021; He *et al.,* 2024). Through processes such as immobilization and mineralization, as well as nitrogen fixation, phosphate solubilization, and organic matter mineralization, soil microorganisms play a key role in ensuring adequate plant nutrition. They help reduce reliance on chemical fertilizers and are crucial in regulating the carbon cycle, contributing to the capture and storage of organic carbon in the soil (Moore *et al.,* 2020; Pokharel *et al*., 2020; Zhu *et al*., 2020; Wen *et al*., 2023; Huang *et al*., 2024). Given these vital functions, understanding and promoting the health of the soil microbiome is essential for sustainable agriculture. Investing in agricultural practices and technologies that enhance soil microbial diversity not only boosts crop productivity but also supports the conservation of natural resources, ensuring long-term food and environmental security. At present, global agriculture is being transformed by the innovations offered by companies in the market. New technologies are influencing every aspect of agriculture, from machinery to sustainable management practices (Zhang *et al*., 2024). This includes advancements in soil microbiology. Among the technologies directly linked to regenerative agriculture and food production is Penergetic technology. Penergetic technology represents an innovation in agriculture and environmental management. Developed based on principles of bioenergy and biophysics, this technology employs special formulations to improve soil health, promote plant growth, and enhance agricultural productivity through magnetic and biophysical stimuli in microbial and plant cells (Ernstfried, 2009). Unlike many conventional approaches, Penergetic technology provides more than just direct benefits to plants; it takes a holistic approach by stimulating biological processes in the soil and strengthening interactions between the soil, plants, and microorganisms (Lertpatarakomol *et al*., 2017; Sharib *et al*., 2020; Sharifi *et al*., 2020; Artyszak *et al*., 2021; Hata *et al*., 2021; Butkevičienė *et al*., 2023; Goes *et al*., 2023). According to the manufacturer of Penergetic technology, it promotes beneficial microbial activity in the soil, optimizes water and nutrient use, enhances plant resistance to both biotic and abiotic stresses, and reduces nutrient leaching. As a result, farmers can achieve higher crop yields while contributing to

the conservation of natural resources and the sustainability of agricultural systems (Sharib*et al*., 2020; Goes *et al*., 2023). Given that stimulating microorganisms in the soil is a fundamental aspect of improving the quality of production systems, the objective of this study was to evaluate the effects of Penergetic "p" on the stimulation of the development and multiplication of microbial agents commonly used in global agriculture.

MATERIAL AND METHODS

The tests were conducted in the soil microbiology laboratory of BioTecRS (Santa Maria, Rio Grande do Sul, Brazil) to evaluate the effectiveness of the biostimulant (Penergetic "p") in stimulating the growth and development of microbial agents in vitro. The biostimulant is produced by Penergetic International AG (Romanshorn, Switzerland). The microbial agents evaluated included the bacteria *Azospirillum brasilense*, the fungi *Beauveria bassiana* and *Isaria fumosorosea* (formerly *Cordyceps fumosorosea*), bacterial species from the *Bacillus* genus (*B. amyloliquefaciens*, *B. aryabhattai*, *B. licheniformis*, *B. methylotrophicus*, and *B. subtilis*), and fungal species from the *Trichoderma* genus (*T. asperelloides*, *T. asperellum*, *T. harzianum*, and *T. virens*).

These microbial agents were isolated from commercial products, and their pure cultures were maintained on Potato-Dextrose-Agar (PDA) culture medium. The tests to evaluate microbial growth with and without the biostimulant were carried out using both liquid and semi-solid fermentation processes. In the liquid fermentation process, 120 mL Erlenmeyer flasks containing 50 mL of Potato-Dextrose culture medium were used. For the semi-solid fermentation process, 90 mm diameter glass Petri dishes with 30 mL of Potato-Dextrose-Agar culture medium were used. For both fermentation processes, each microorganism or combination of microorganisms was tested in two treatments: one without the biostimulant (control treatment) and one with the biostimulant (treatment with the addition of 1.5% Penergetic "p" relative to the volume of the culture medium, either liquid or semi-solid). The biostimulant was added to the culture medium prior to sterilization in an autoclave at 1 atm and 120°C for 20 minutes.

After the culture medium was naturally cooled, a 9 mm diameter disc of each microorganism was used as the initial inoculum. The inoculum was added to the liquid medium (liquid fermentation) or deposited in the center of each Petri dish (semi-solid fermentation), with 12 replicates for each treatment.

The experimental units were maintained in a microbial growth chamber at 25°C with a 12-hour photoperiod. Bacterial growth was assessed 96 hours after incubation, while fungal growth was assessed 120 hours after incubation. For both bacterial and fungal agents, the number of spores per milliliter of culture medium was counted using a Neubauer chamber and an optical microscope with 400x magnification. Growth on solid culture medium was recorded to compare the growth rates between the control and Penergetic biostimulant treatments for each microbial agent evaluated. The results were transformed into percentages to quantify the stimulatory effect of the biostimulant for each treatment. The microbial growth data obtained were subjected to statistical analysis, and means were compared using the SISVAR statistical software (Ferreira, 2011).

RESULTS AND DISCUSSION

A microbial stimulatory effect was observed with the biostimulant product at a concentration of 1.5% in the culture medium for all microbial agents evaluated. The most pronounced stimulatory effect was seen in bacterial agents, except for the fungus *Isaria fumosorosea*. Fungi from the *Trichoderma* genus and the species *Beauveria bassiana* showed a weaker response to the biostimulant stimulus (Table 1). A species-specific response to the biostimulant was observed in promoting microbial growth. Among the *Bacillus* species, *B. methylotrophicus* and *B. licheniformis* exhibited the most intense response to the stimulus, with multiplication rates increasing by approximately 297.29% and 122.37%, respectively. Among the *Trichoderma* species, *T. asperellum* demonstrated the highest percentage of stimulation, with an increase in fungal agent multiplication of 46.66% (Table 1). Research on the effect of in vitro microbial stimulation has consistently demonstrated the significant benefits of using the biostimulant Penergetic "p" and its role in enhancing beneficial microbial activity in the soil (Artyszak *et al*., 2020; Steffen *et al*., 2020; Makaveckas *et al*., 2021; Marques *et al*., 2022). Studies have shown that the application of stimulating agents such as specific organic compounds, extracts of selected microorganisms, and biophysical and magnetic stimulation technologies like Penergetic can increase the diversity and metabolic activity of soil microorganisms. These include nitrogen-fixing bacteria, mycorrhizae, phosphate-solubilizing microorganisms, and decomposer organisms (Franco Júnior *et al*., 2019; Steffen *et al*., 2020; Kerdokas *et al*., 2020).

These positive effects are often linked to improvements in plant nutrient availability, increased fertilizer use efficiency, and enhanced plant resistance to biotic and abiotic stresses. The results underscore the promising potential of promoting the soil microbiome as an effective strategy to optimize soil health and improve agricultural system sustainability. In agriculture, it is common to use combinations of microorganisms, such as two species from the same genus in a single product. Among the most used combinations are bacteria from the *Bacillus* genus and fungi from the *Trichoderma* genus, which are widely marketed as plant growth promoters or for plant pathogen control. The results of this study demonstrate that the Penergetic "p" product is an effective tool for enhancing the activity of these microbial agents, thereby improving the efficiency of microorganisms applied in the field. Stimulating microbial activity favors their establishment in field conditions, enabling microorganisms to fully express their agricultural and environmental potential, thus benefiting plant production processes. The microbial growth results obtained in semi-solid fermentation (culture medium) in terms of bacterial and fungal colony diameter showed superior microbial growth stimulation for bacterial agents compared to the fungal agents evaluated (Table 2). The stimulation was more intense in relation to the growth area (diameter of bacterial colonies) for bacteria (Figure 1). In fungi, particularly those of the *Trichoderma* genus, the response to the stimulus was more pronounced during sporulation (conidia maturation) (Figure 2). Given that microbial agents applied to the soil in the form of bioinputs must first establish themselves in the soil environment before expressing their full potential (in promoting plant growth, bioprotection, and biosulbilization), accelerating microbial growth enhances the conditions necessary for effective

Table 1 - Effect of stimulating microbial multiplication *in vitro* observed in the control and biostimulanttreatments on microbial agents multiplied by liquid fermentation

Microorganism	Control (Number in millions of spores)	Biostimulant (Number in millions of spores)	Biostimulation (Stimulus effect)	CV (%)
Azospirillum brasilense	132.80B[2]	665.73A	401.31%	1.62
Bacillus amyloliquefaciens	13.00A	22.5A	73.07%	15.06
Bacillus methylotrophicus	37.00B	147A	297.29%	6.33
Bacillus subtilis	8.50B	15.45A	81.76%	16.54
Bacillus aryabhattai	12.10B	22.05A	82.23%	14.19
Bacillus licheniformis	9.50B	21.17A	122.37%	27.07
Trichoderma asperelloides	85.20A	103.63A	21.63%	9.62
Trichoderma asperellum	84.54B	124A	46,66%	4.82
Trichoderma harzianum	82.30B	101.5A	23.32%	5.98
Trichoderma virens	65.70B	88.19A	34.23%	4.10
Beauveria bassiana	85.90B	118.59A	38.05%	3.59
Isaria fumosorosea[1]	121.60B	306.30A	151.89%	18.22

[1]Synonym of *Cordyceps fumosorosea.;* [2]Means followed by the same letter in the rows do not differ according to Tukey's test at a 5% probability level.

Table 2. Effect of stimulating microbial growth *in vitro* observed in control and biostimulant treatments on microbial agents multiplied by semi-solid fermentation

Microorganism	Control (growth area in mm^2)	Biostimulant (growth area in mm^2)	Biostimulation (Stimulus effect)	CV (%)
Azospirillum brasilense	3508.44B[2]	17122.23A	388,03%	2.84
Bacillus amyloliquefaciens	2123.72B	5808.80A	173.52%	21.14
Bacillus methylotrophicus	2341.08B	6991.20A	198.54%	8.01
Bacillus subtilis	2018.79B	3535.10A	75.11%	14.66
Bacillus aryabhattai	2202.93B	3942.14A	78.95%	17.25
Bacillus licheniformis	2545.36B	5687.35A	123.44%	25.06
Trichoderma asperelloides	19040.01B	24251.26A	27.37%	13.61
Trichoderma asperellum	17254.52B	25074.26A	45.32%	5.89
Trichoderma harzianum	18089.93B	21901.47A	21.07%	6.34
Trichoderma virens	17555.23B	23337.92A	32.94%	5.88
Beauveria bassiana	10117.56B	13303.65A	31.55%	4.27
Isaria fumosorosea[1]	11532.81B	24378.05A	111.38%	15.49

[1]Synonym of *Cordyceps fumosorosea.;* [2]Means followed by the same letter in the rows do not differ according to Tukey's test at a 5% probability level.

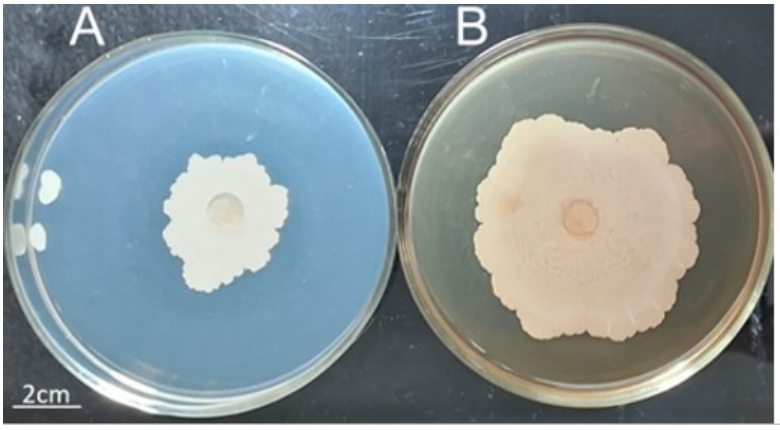

Figure 1. Growth of the bacteria *Bacillus amyloliquefaciens* with 96 hours of incubation in solid culture medium in the control (A) and biostimulant (B) treatments

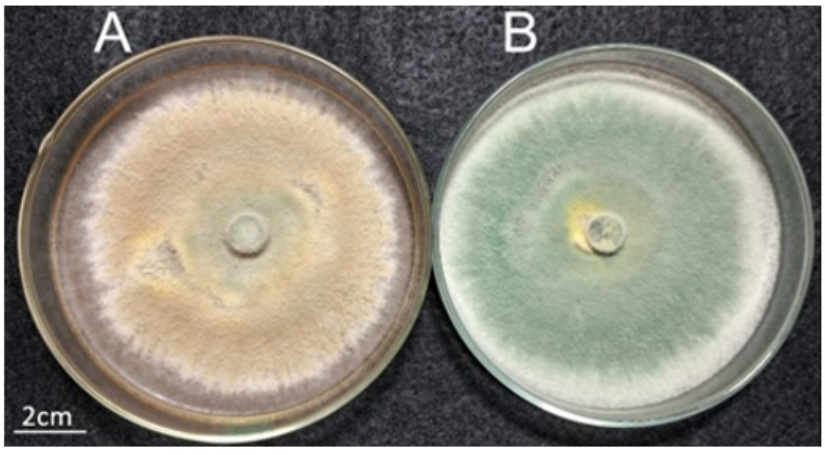

Figure 2. Growth of the fungus *Trichoderma harzianum* with 120 hours of incubation in solid culture medium in the control (A) and biostimulant (B) treatments

microbial interaction and activity within the soil/plant system after application. In this context, biological stimulation alternatives are crucial tools for optimizing microbial symbioses in agriculture. The effect of biophysical stimuli on microbial activity is a complex phenomenon involving interactions between microorganisms and their physical environment. Several biophysical factors, such as temperature, pH, pressure, radiation, and vibration, can significantly influence the rate and nature of microbial metabolic activities (Larson, 2023; Chai *et al*., 2024). Among these factors, energy can accelerate enzymatic reactions, which in turn increases the rate of microbial growth. This acceleration leads to modifications in protein structure, thereby influencing microorganisms' ability to carry out essential functions. Additionally, exposure to specific frequencies and vibrations can induce either beneficial or harmful changes in microorganisms, resulting in alterations to their metabolic behavior. The use of vibrational energy as a microbial stimulator is an innovative approach that has generated increasing interest in microbiological research and biotechnology (Buffi *et al.*, 2025). Vibrational energy, particularly within the infrasound or ultrasound ranges, can directly influence the metabolic activities and growth of microorganisms. The controlled application of these vibrations can enhance the permeability of cell membranes, facilitating the entry of water and nutrients, and improving the efficiency of metabolic processes, such as the production of industrial or biologically relevant compounds (Hu *et al*., 2023; Xu *et al*., 2024).

In-depth studies on the use of vibrational energy as a microbial stimulator are highly relevant, as this stimulus represents a promising research frontier with the potential to revolutionize several biotechnological and environmental applications. Microbial dynamics in agricultural soils is a complex and crucial process for the health and productivity of agricultural systems. Agricultural soils are home to a rich diversity of microorganisms that interact with one another and with plant roots, forming intricate trophic networks and regulating biogeochemical cycles. As such, understanding microbial dynamics in agricultural soils and recognizing the importance of maintaining soil biological activity are key to developing sustainable agricultural practices and promoting soil health and crop productivity.

CONCLUSION

Microbial agents widely used as bioinputs in agriculture were stimulated by the biostimulant product Penergetic “p” (produced by the Swiss company Penergetic). The addition of the biostimulant to the microorganism cultivation medium accelerated the activity and growth rate of both fungal and bacterial structures in the evaluated microorganism species. The biostimulation effect of Penergetic “p” varied depending on the species of microorganism. The highest percentage of biostimulation was observed for the bacterium *Azospirillum brasilense* (401.31%), followed by *Bacillus methylotrophicus* (297.29%). Among the other three *Bacillus* species, the biostimulation percentages ranged from 122.37% for *B. licheniformis* to 82% for *B. subtilis* and *B. aryabhattai*. Fungal agents showed less intense biostimulation effects with the addition of Penergetic “p” compared to bacterial agents, except for the fungus *Isaria fumosorosea*, which exhibited a 151.89% increase in growth due to the biostimulant.

For the four species of the *Trichoderma* genus, the percentages of biostimulation ranged from 23% to 46%, with the highest being for *T. asperelloides* (46%) and the lowest for *T. harzianum* (23%). The multiplication of *Beauveria bassiana* was stimulated by 31.55% with the addition of Penergetic “p” to the culture medium. Based on the results of this study, there is strong evidence supporting the benefits of using Penergetic “p” to enhance the growth and multiplication of key agricultural bioinput agents.

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Data Availability: The datasets generated during the current study are available from the corresponding author on reasonable request.

REFERENCES

Artyszak, A.; Darjusz, G. (2020). The effect of growth activators and plant growth-promoting rhizobacteria (PGPR) on the soil properties, root yield, and technological quality of sugar beet. *Agronomy*, *10*(9), 1262, 2020.https://doi.org/10.3390/agronomy10091262.

Artyszak, A.; Gozdowski, D. (2021). Application of growth activators and Plant Growth-Promoting Rhizobacteria as a method of introducing a “farm to fork” strategy in crop management of winter oilseed. *Sustainability*, *13*(6), 3562, 2021. https://doi.org/10.3390/su13063562.

Buffi, M. *et al*. (2025). Electrical signaling in fungi: past and present challenges. *FEMS Microbiology Reviews*, fuaf009. https://doi.org/10.1093/femsre/fuaf009.

Butkevičienė, L. M.*et al*. (2023). Effect of Different Tillage Systems and Soil Biostimulantson Agrochemical Properties and Intensity of Soil CO_2 Emission in Wheat Crop. *Agronomy*, v. *13*(2), 338, 2023. https:// doi.org/10.3390/ agronomy13020338.

Cao, Y. *et al*. (2021). Organic-C quality as a key driver of microbial nitrogen immobilization in soil: A meta-analysis. *Geoderma*, *383*, 114784, https://doi.org/10.1016/j.geoderma.2020.114784.

Chai, L.; Shank, E. A.; Zaburdaev, V. (2024). Where bacteria and eukaryotes meet. *Journal of Bacteriology*, *206*(2), e00049-23. https://doi.org/10.1128/jb.00049-23.

Ernstfried, P. (2009). *A Vision Becomes Reality*: A Forward-Looking Technology enables us to act in an Economical and Ecological Manner for the Benefit of Humans, Animals and the Environment. Hardcover, 200p.

Feng, J. *et al*. (2022). Changes in plant inputs alter soil carbon and microbial communities in forest ecosystems. *Global Change Biology*, *28*(10), 3426-3440, https://doi.org/ 10.1111/gcb.16107.

Ferreira, D. F. (2011). Sisvar: um sistema computacional de análise estatística. *Ciência e Agrotecnologia*, *35*(6), 1039-1042,https://doi.org/10.1590/S1413-70542011000600001.

Franco Júnior, K. S. *et al.* (2019). Influence of the use coverage plants and the bioactivator in the physical-biological characteristics of soil cultivated with coffee. *Coffee Science, 14*(1), 123-126, https://doi.org/ 10.25186/ cs. v14i1.1553.

Goes, G. B. de. *et al.* (2023). Bioactivator, phosphorus and potassium fertilization and their effects on soil, physiology, production and quality of melon. *Acta Physiologiae Plantarum*, *45*(4), 1-14, https://doi.org/10.1007/s11738-023-03536-5.

Hata, F. T. *et al*. (2021). Bokashi, boiled manure and penergetic applications increased agronomic production variables and may enhance powdery mildew severity of organic tomato plants. *Horticulturae*, *7*(2), 27, https://doi.org/10.3390/horticulturae7020027.

He, M. (2024). Understanding the stimulation of microbial oxidation of organic N to nitrate in plant soil systems. *Soil Biology and Biochemistry*, *190*, 109312, https://doi.org/10.1016/j.soilbio.2024.109312.

Hu, X.; Ouyang, T. (2023). An integrated assessment of microfluidic microbial fuel cell subjected to vibration excitation. *Applied Energy*, *336*, 120852, https://doi.org/ 10.1016/j.apenergy.2023.12085.

Huang, B. L. X. *et al.*(2024). Microbial organic fertilizer prepared by co-composting of Trichoderma dregs mitigates dissemination of resistance, virulence genes, and bacterial pathogens in soil and rhizosphere. *Environmental Research*, *241*, 117718,https://doi.org/10.1016/j.envres.2023.117718.

Kerdokas, T. (2020). Biologinėskilm ėspreparatųir jųmišini ųnaudojimoįtakadirvožemiosavybėms. *Kaunas,* 113-116. https://doi.org/10.7220/1822-9913.2020.

Larson, B. T. (2023). Perspectives on Principles of Cellular Behavior from the Biophysics of Protists. *Integrative and Comparative Biology*, *63*(6), 1405-1421, https://doi.org/10.1093/icb/icad106.

Lertpatarakomol, R. *et al.* (2017). Effects of Penergetic fresh wave at different levels for extending the shelf life of sausage. Nurturing Locally, *Growing Globally*, *414*, https://doi.org/10.3920/9789086868605_183.

Ma, X. *et al.* (2021). Soil microbial community succession and interactions during combined plant/white-rot fungus remediation of polycyclic aromatic hydrocarbons. *Science of The Total Environment*, *752*, 142224, https: //doi.org/10.1016/j.scitotenv.2020.142224.

Makaveckas, M. (2021). Biologinės kilmės preparatų ir tarpiniopasėlioįtakaseptoriozėspaplitimui. *AgronomijosFakultetas*, 17. https://doi.org/10.7220/1822-9913.2021.

Marques, K. R. *et al*. Phosphate fertilization and bioactivator influences on fractions of organic matter and soil microbial biomass. *Research, Society and Development*, *11*(9), https://doi.org/10.33448/rsd-v11i9.32086.

Moore, J. A. *et al*. (2020). Plant roots stimulate the decomposition of complex, but not simple, soil carbon. *Functional Ecology*, *34*(4), 899-910, https://doi.org /10. 1111/1365-2435.13510.

Pokharel, P., Ma, Z., Chang, S. X. (2020). Biochar increases soil microbial biomass with changes in extra-and intracellular enzyme activities: a global meta-analysis. *Biochar*, *2*, 65-79. https://doi.org/10.1007/s42773-020-00039-1.

Sharib, B. *et al*. (2020). Evaluation of alfalfa traits by using penergetic P and K under free and Infested by Dodder (*Cuscuta campestris*). *Journal of Agricultural Science and Sustainable Production, 30(*2), 177-189.

Sharifi, M. *et al*. (2020). Evaluation of grain and essential oil yield of cumin (*Cuminum cyminum* L.) using of penergetic, chemical fertilizers and magnetic Water. *Journal of Agricultural Science and Sustainable Production*.*30*(1), 141-153.

Steffen, R. B. *et al*. (2020). In vitro activation of microbial growth in low frequency electromagnetic fields. *Journal of Agriculture and Environmental Sciences*, *9*(1), 1-7. https://doi.org/10.15640/jaes.v9n1a1.

Wen, T. *et al*. (2023). Deciphering the mechanism of fungal pathogen-induced disease-suppressive soil. *New Phytologist*, *238*(6), 2634-2650. https://doi.org/ 10.1111/ nph.18886.

Xu, Y. *et al*. (2024) Electrocatalytic denitrification biofilter for advanced purification of chlorophenols via ceramsite-based Ti/SnO2–Sb particle electrode: Performance, microbial community structure and mechanism. *Environmental Pollution*, 123594. https://doi.org/10.1016/j.envpol.2024.123594 .

Zhang, R.*et al.* (2024). Manipulating soil microbial community assembly by the cooperation of exogenous bacteria and biochar for establishing an efficient and healthy CH4 biofiltration system. *Chemosphere*, *352*, 141319, https://doi.org/10.1016/j.chemosphere.2024.141319.

Zhu, X. *et al*. (2020). The soil microbial carbon pump: From conceptual insights to empirical assessments. *Global Change Biology*, *26*(11), 6032-6039.https:// doi.org/1 0.1111/ gcb.15319.

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