Botanical Studies

ORIGINAL ARTICLE

Open Access

Coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), larval frass as plant fertilizer

Chiao-Jung Han[1,2], Zeng-Yei Hseu[3], Po-Hui Wu[3], Louis Grillet[3], Chun-Han Ko[4] and Matan Shelomi[1*]

Abstract

Background Beetle rearing for food or feed is a growing area of agriculture that produces considerable wastes. This frass is a putative soil amendment anecdotally applied directly as fertilizer. To determine if beetle waste can be used as a soil amendment without pre-treatment, a series of chemical, physical, microbiological, and plant-growth assays were performed on waste from the coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), fed cocopeat. Beetle diet and frass NPK levels, C:N ratio, and particle size were measured. Microbiota was identified with metabarcoding, and functional profile analysis done to identify pathways associated with wood digestion or plant growth. Cultivation tests were done with *Arabidopsis thaliana* (Brassicales: Brassicaceae) and frass incorporation into potting soil at 0, 20, or 40%, followed by elemental concentration measurement of the soil, frass, and plant matter.

Results Digestion of plant polysaccharides in the gut, primarily by microbial depolymerizers, produces frass of uniform particle size and NPK 1.8–0.13–1.2 that can be used directly as a mature fertilizer without pre-composting, or even as a growth substrate. Plants with higher proportions of frass in the soil grew significantly faster and larger compared to a nutrient-rich potting soil. Frass is high in useful elements and has beneficial chitinolytic microbes.

Conclusions Beetle frass can thus be used directly as a soil amendment without composting or pretreatment, with positive effects on plant growth even compared to rich soils. Valorizing frass in this way generates income for beetle farmers and recycles nutrients to soil as part of circular agriculture.

Keywords Elemental analysis, Fertilizer, Frass, Microbiome, Rhinoceros beetle, Soil amendment

*Correspondence:
Matan Shelomi
mshelomi@ntu.edu.tw
[1] Department of Entomology, National Taiwan University, Taipei, Taiwan
[2] Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan
[3] Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan
[4] School of Forestry & Resource Conservation, National Taiwan University, Taipei, Taiwan

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Background

Animal manure has a long history of use as fertilizer, including invertebrate manure such as earthworm castings. Recent data suggests the frass of farmed insects has high fertilizing index values, capable of enhancing plant defenses under biotic or abiotic stress and providing more plant accessible nutrients (Lovett and Ruesink 1995; Barragán-Fonseca et al. 2022; Blakstad et al. 2023; Poveda et al. 2019; Watson et al. 2021), although some species' frass requires prior composting to mature (Beesigamukama et al. 2022). These studies have been predominantly on frass from the three most commonly farmed insects (Davidowitz 2021): mealworms (Blakstad et al. 2023; Poveda et al. 2019; Wantulla et al. 2022), black soldier fly (Wantulla et al. 2022; Wang et al. 2022; Lopes et al. 2022), and house cricket (Wantulla et al. 2022). However, other species are or can be farmed more sustainably in certain habitats than these (Davidowitz 2021). Their frass too warrants investigation, as valorizing this waste could potentially bring more profits to their farmers, especially small-scale and developing world farmers (Beesigamukama et al. 2022).

Among the beetles (Coleoptera), several species besides mealworms (Tenebrionidae), are already reared commercially. This includes the palm weevil larvae (Curculionidae) reared as food in the tropics (Cito et al. 2017), and the long-horned (Cerambycidae), rhinoceros (Scarabaeidae: Dynastinae), and stag beetles (Lucanidae) reared in East Asia for trade and as pets. Beetles can produce prodigious amounts of waste: the frass of leaf-defoliating beetles was found to contribute significant amounts of nitrogen to the soil and lead to greater nitrogen levels in plant tissues (Uriarte 2000; Gherlenda et al. 2016). Informally, beetle breeders do give their beetle frass to farmers for use as fertilizer. However, with the exception of mealworm frass, the potential risks and benefits of using beetle frass as a soil amendment have not been rigorously analyzed. In fact, only one publication has looked at other beetles' frass, specifically the African fruit beetle (*Pachnoda sinuata* L.) and the coconut rhinoceros beetle (*Oryctes rhinoceros* L.); both are scarab beetles whose frass showed promising results (Beesigamukama et al. 2022).

This study sought to determine with greater detail the prospects for *Oryctes rhinoceros* frass use as a soil amendment or fertilizer. The species was chosen as it is a pest on palm trees (Pradipta et al. 2020) that nonetheless has potential as a food (Bolaji et al. 2021) and frass source (Beesigamukama et al. 2022), and is an easy-to-rear, inexpensive model for the Scarabaeidae beetles. Open questions about the frass include the impact of its microbiome on plant growth. Insect frass can transmit microbes the insects obtained from their diet, which could include plant pathogens as well as beneficial microbes (Poveda 2021; Osimani et al. 2018). Other potential effects of the microbiome include phytohormone synthesis, nitrogen fixation, phosphorus solubilization, sulfur oxidation, and plant cell wall depolymerization. The *O. rhinoceros* larval gut contains a complex microbiome capable of digesting wood polysaccharides such as cellulose, hemicellulose, and lignin (Han et al. 2024). The effects of these microbes in the frass when applied as a plant fertilizer are unknown.

Phytotoxicity from application of immature compost is another issue. The prior study on *Oryctes rhinoceros* and other insects' frasses as a soil amendment found the frasses had sufficient values for indices like pH, C/N ratio, and ammonium/nitrate ratio to qualify as a mature compost, but the *O. rhinoceros* frass had moderate phytotoxicity and "minimal suitability for crop production" (Beesigamukama et al. 2022). However, anecdotal use of *O. rhinoceros* frass as a fertilizer suggests that conclusion is not universal.

The goals of this study were to characterize *O. rhinoceros* frass, determine what nutrients it could provide to plants, what microbes it contains, and whether it can be used directly as a soil amendment without pre-treatment. We hypothesized based on its use by farmers that there would be minimal to no phytotoxicity from the frass, and that its nutrient quality and/or microbial components could provide measurable benefits to plants grown in soil with added frass.

Materials and methods

Beetle rearing

Oryctes rhinoceros beetles collected from decaying palm logs in southern Taiwan were maintained in the Department of Entomology laboratory for one generation on a diet of commercial cocopeat (*Cocos nucifera*, Arecales: Arecaecae) (AgroBrothers Coir Company, India) supplemented with shredded areca palm wood (*Areca catechu*, Arecales: Arecaecae). The frass from third instar larvae was collected by sifting through a sieve, and stored in a sealed plastic bag to reduce loss of nitrogen in a refrigerator to prevent decay. Approximately 500 g of frass could be produced by ten beetle larvae over the course of one week.

Chemical and physical analysis

The effects of beetle biocomposting on nutrient concentrations were investigated. Samples of beetle frass, commercial cocopeat, and the shredded wood were homogenized and analyzed, including NPK levels and C:N ratio. All air-dried samples were ground to pass through a 20 mesh sieve, heated at 70 °C for 24 h to inhibit enzyme activity, and stored in plastic bags prior

to analysis. To determine the moisture content, two grams of samples were oven-heated at 105 °C for 5 h and weighed. The mineral content was determined as followed. For dry ashing, two grams of samples in crucibles were placed in a furnace at 250 °C for 1 h, and then at 550 °C for 4 h, after which the samples were removed from the furnace and cooled down. The samples were weighed and placed in the furnace again until their weight remained constant. After calculating the percentage of moisture and minerals, the sum of the two were subtracted from 100% to get deduct the percentage of organic matter (Nelson and Sommers 1982). The total nitrogen content was determined by the Kjeldahl method (Bremner 1960). The pH of the sample was determined by a LAQUA F-71 glass electrode (HORIBA Advanced techno Co., Ltd.) with the ratio of sample/water in 1:5 (McLean 1982). The total contents of P, K, Fe, Mn, Cu, and Zn, were measured by portable x-ray fluorescence (pXRF) using an Olympus DP-2000-*C* Delta Premium Alloy XRF Analyzer. Statistical analysis was performed using one way ANOVA followed by a post hoc Tukey HSD Test using an online calculator (https://astatsa.com/).

Particle size distribution and shape analysis was used to observe the impact of beetle larvae digestion on the physical properties of the cocopeat (Holzinger et al. 2023). For this, 100 g of cocopeat and 100 g of frass dissolved in water and sonicated into separate particles were analyzed using a Microtrac Flow Synchronous Laser Diffraction machine (Microtrac MRB, Montgomeryville, PA, USA) and Dynamic Image Particle Analyzer (Microtrac MRB, Montgomeryville, PA, USA).

Microbiome analysis

As soil or compost microbiota can stimulate plants to make a beneficial lignin barrier on the roots, microbial analysis was performed. Three biological replicates each were sampled from the lived-in larval rearing substrate or "beetle soil," the cocopeat direct from the package, larval fecal pellets taken from the larval containers ["old frass"], and fresh larval fecal pellets coaxed directly from the larvae into a sterile collection tube ["fresh frass"]. Their DNA was extracted using a PrestoTM Soil DNA Extraction Kit (Geneaid) according to the manufacturer's instructions. The extracted DNA was pre-purified using the OneStep PCR Inhibitor Removal Kit (Zymo Research) and then purified with a DNeasy® PowerClean® Pro Cleanup Kit (QIAGEN). The purified DNA samples were sent to BioTools Co. Ltd. (Taiwan) for amplification sequencing of the hypervariable V3 and V4 regions of the 16S rDNA using paired-end Illumina Miseq (300 bp paired-end reads). The sequencing data have been deposited in NCBI GenBank (Bioproject: PRJNA1052367). Following sequencing, QIIME2 v2023.9 (Bolyen et al. 2019; Estaki et al. 2020) was used with default parameters for quality filtering and microbiome analysis as reported previously (Shelomi and Chen 2020). Alpha diversity was measured as Shannon, Faith PD, and Pielou's evenness with Kruskal–Wallis and pairwise Kruskal–Wallis tests using QIIME2. Beta diversity by Bray–Curtis was compared with Permanova and pairwise Adonis tests using the *vegan, pairwiseAdonis,* and *ggplot2* packages in R (Oksanen 2017). An NCBI RefSeq classifier for prokaryotic 16S *rRNA* (33175[Bioproject] OR 33317[Bioproject]) was generated using RESCRIPt (Robeson et al. 2021; O'Leary et al. 2016; Tatusova et al.

Table 1 The functional groups with enzymes/genes for PICRUSt2 analysis

Functional group	Enzymes/genes were searched
Auxin synthesis	Amine-oxidase, tryptophan transaminase, *dhaS, iaaH,* IAAld dehydrogenase, *iaaM*, IAM-hydrolase, *IPdC*, nitrilase, nitrilehydratase, *pat*B, trp decarboxylase, *trpA, trpB, trpD, trpE,* tryptophan side-chain oxidase, *yclB, yclC, yhcX, ysnE* (Keswani et al. 2020; Spaepen and Vanderleyden 2011)
Cytokinin synthesis	Beta-glucosidase, *CKX, CYP450, FasD, iPRMP, ipt, LAS, LDCs, LOG, MiaB, MiaE, N-GT, NoIPT1, Tmr, tZRMP,Tzs, ZI, ZOGT, ZR* (Frébort et al. 2011; Frébortová and Frebort 2021; Seo et al. 2016)
Nitrogen fixation	*amtB, AnfG, draG, draT, fixA, fixB, fixC, fixJ, fixK, fixL, fixL-fixJ, fixR, fixT, fixX, glnB, glnK, nifA, nifB, nifD,nifE, nifH, nifK, nifL, nifN, nifP,nifQ,nifS, nifT, nifU, nifV, nifW, nifX, nifZ, nrf1,NtrB, NtrB-NtrC, NtrC, RegB, RegR, RegS-RegR, VnfG* (Dixon and Kahn 2004; Santos et al. 2012; Dai et al. 2014)
Phosphorus solubilization	*acpA, aphA, appA, appA2, gabY, gcd, mps, napA, napD, napE, olpA, pcc, phnC, phnD, phnE, phnG, phnH, phnI, phnJ, phnL, phnM, phnN, phnO, phnP, phnW, phnX, phoA, phoB, PhoC, phoD, phoN, phoR, phoX, phy, phyA, ppa, ppx, pstA, pstB, pstC, pstS, ugpA, ugpB, ugpC, ugpE, ugpQ* (Rodríguez et al. 2006; Liang et al. 2020)
Sulfur oxidation	*APR, AprBA, DoxDA, DsrA, DsrB, DsrC, DsrE, DsrE2B, DsrE3, DsrE3A, DsrEFH, DsrF, DsrH, DsrJ, DsrK,DsrM, DsrO, DsrP, FCC, Hdr,Rhd, SAT, SDH, SoxB, SoxCD, SoxXA, SoxY, SoxYZ, SoxYZXA, SoxZ, SQR, TcDH, Tqo, TusA* (Dahl 2015; Friedrich et al. 2005)
Cell wall and exoskeleton depolymerization	Plant cell wall degrading enzymes as recorded on dbCAN2 web server (https://bcb.unl.edu/dbCAN2/) (Zhang et al. 2018) including those from auxiliary activities, carbohydrate esterase, glycosidase hydrolase, polysaccharide lyase families, etc
Chitin degradation	As recorded on dbCAN2 web server (Zhang et al. 2018)

2016) for the taxonomic assignments. Functional profile analysis was used to see the potential bio-pathways or functions provided by microbes in the *O. rhinoceros* frass. This was done using the standard pipeline of PICRUSt2 v2.4.1 (Douglas et al. 2020) on the online Nephele:Microbiome web server of U.S. National Institutes of Health & National Institute of Allergy & Infectious Diseases (https://nephele.niaid.nih.gov/init_picrust2) for KEGG orthology prediction of genes associated with the following: synthesis of plant growth hormone (auxins and cytokinins); nitrogen fixation, phosphorus solubilization, and sulfur oxidation pathways; and plant cell wall and exoskeleton depolymerization (plant cell wall degrading enzymes and chitinases) (Table 1). To investigate microbial composition differences among the four sample types (cocopeat, beetle soil, fresh frass collected directly from the larva, old frass collected from the substrate), a non-metric multidimensional scaling (nMDS) plot was utilized to visually represent clustering patterns, along with corresponding statistical analyses.

Cultivation test and determination of elemental concentrations

To determine the effect of fertilization with beetle frass on plant growth, the standard laboratory model plant *Arabidopsis thaliana* (L.) Heynh accession Columbia (Col-0) (Somssich 2019) (Brassicales: Brassicaceae) was grown in a climate chamber at 22 °C (16 h light/8 h dark). Plants were irrigated once a week with demineralized water. The soil consisted of a mix of four parts of potting soil (Jiffy®, The Netherlands), one part of perlite, and one part of vermiculite; and fresh frass was incorporated or not to the mix at 0%, 20%, or 40% w/w. The same ratios or higher (up to 50%) have been used in published studies of insect frass as a soil amendment (Tan et al. 2021; Song et al. 2021; Houben et al. 2020). One replicate consisted of six pots of each treatment, and the 18 pots of each experiment were placed randomly in a 10 ×20-inch tray. The experiment was carried out in three independent replicates. When the last plant started flowering, which happened at seven weeks after sowing, the height of the inflorescences was measured, the plants were harvested, and their fresh and dry weight was recorded individually. Statistical analysis was done by with a one way ANOVA followed by a Duncan test with $p < 0.05$ using R's *agricolae* package (de Mendiburu and Yaseen 2020).

To measure the elemental concentrations in the plant tissues, the harvested plants were washed twice in 10 mM $CaCl_2$ and 1 mM EDTA and twice with ultrapure water. Each batch of six plants were dried in an oven at 60 °C for 72 h. Ten mg of dry tissues were mineralized in 65% HNO_3 in a microwave, homogenized with mortar and pestles, and diluted in 10 mL ultrapure water before analysis with an Agilent 7800 ICP-MS. A certified sample of tomato leaves was also analyzed to evaluate the accuracy. Each sample was processed and analyzed in duplicate. Statistical analysis was done by with a one way ANOVA followed by a Duncan test with $p < 0.05$ using R's *agricolae* package (de Mendiburu and Yaseen 2020).

An elemental concentration test was also done to compare the potting soil and the frass. For each, a 0.2 g sample was mixed with 6 mL of HNO_3 in a Teflon vessel and left to react for 14 h. The sample was then pre-digested by heating to 100 °C over 10 min in a microwave oven (Speedwave Entry, Berghof, Eningen, Germany). Subsequently, 2 mL of 35% H_2O_2 was added, and the temperature was gradually increased to 120 °C over eight minutes, where it was held for two minutes. The temperature was further raised to 160 °C within five minutes and maintained for another five minutes. In the final stage, it was increased to 180 °C over five minutes and held for 15 min. After cooling, the digested solution was diluted to 10 mL with 2% HNO_3 and filtered through a 0.45 µm Millipore filter. The elemental concentrations for Al, B, Ca, Cr, Cu, K, Mg, Mn, Na, and P were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 2100DV, PerkinElmer, Waltham, MA, USA), and the rest with pXRF as described above. Means for frass and soil were compared using a one-tailed, unpaired t-test.

Results

Chemical and physical analyses of beetle diet and frass

The results of the nutrient analyses are in Table 2. The beetle's diet was comprised mostly of cocopeat with areca wood supplementation. No significant differences were found between the levels in the frass and the cocopeat of nitrogen, potassium, copper, and ash. Compared to the initial wood, the beetle frass contained more nitrogen, phosphorus, potassium, iron, and manganese per unit weight, and less carbon, copper, and zinc (Table 2). The pH of the frass was 7.6, compared to the acidic wood at 5.7. The commercial cocopeat's values were in between those of the frass and the wood, except they were higher in phosphorus, potassium, and manganese and lower in zinc, with a pH of 7.8. Whereas the initial wood pulp had an NPK level of approximately 0.9–0.07–0.8 and the cocopeat an NPK of 1.5–0.2–1.3, the frass has an NPK of approximately 1.8–0.13–1.2. The C:N ratio of the frass was the lowest with a mean of 22.8. Supplementary Fig. 1 shows the distribution for the cocopeat particles, which is discreet and noncontinuous as it comes from shredded plant parts, and for the insect frass, which is uniformly and continuously distributed with significantly smaller particles.

Table 2 Inorganic and organic chemical composition of areca nut stalk wood, commercial cocopeat, and the frass from coconut rhinoceros beetles fed this wood

	Insect frass	Areca wood	Cocopeat	$F_{(2)}$ and *p*-value
N (g/kg)	17.7 ± 1.4^{a}	8.8 ± 1.2^{b}	14.9 ± 0.4^{a}	33.56**
P (g/kg)	1.28 ± 0.07^{b}	0.73 ± 0.06^{c}	2.13 ± 0.06^{a}	261.8***
K (g/kg)	12.0 ± 1.0^{a}	7.8 ± 0.2^{b}	12.6 ± 1.1^{a}	17.7*
Fe (g/kg)	3.03 ± 0.07^{a}	0.82 ± 0.06^{c}	2.08 ± 0.10^{b}	409.7***
Mn (mg/kg)	349 ± 5.7^{a}	122 ± 4.2^{c}	281 ± 4.2^{b}	1197***
Cu (mg/kg)	16 ± 1.7^{b}	33 ± 2.4^{a}	11 ± 1.5^{b}	72.4**
Zn (mg/kg)	103 ± 5.4^{b}	165 ± 4.2^{a}	81 ± 3.1^{c}	198.4***
pH	7.6 ± 0^{b}	5.7 ± 0^{c}	7.85 ± 0.07^{a}	1659***
Average % Moisture	11.9 ± 0.06^{a}	9.4 ± 0.1^{b}	10.8 ± 0.1^{c}	318.4***
Average % ash	18.5 ± 0.2^{a}	3.26 ± 0.09^{b}	17.9 ± 0.23^{a}	3518***
% Organic Matter	69.6 ± 0.3^{c}	87.3 ± 0.2^{b}	71.3 ± 0.2^{a}	3969***
% N	1.78 ± 0.14^{a}	0.88 ± 0.12^{b}	1.49 ± 0.04^{a}	33.56**
% Organic C	40.4 ± 0.2^{c}	50.6 ± 0.1^{a}	41.4 ± 0.1^{b}	6415***
NPK	1.8–0.13–1.2	0.9–0.07–0.8	1.5–0.2–1.3	
C:N ratio	22.8 ± 2.0^{b}	58.1 ± 8.1^{a}	27.8 ± 0.9^{b}	31.1**

Data presented is averages of two samples ± standard deviation. pH was measured in samples dissolved 1:5 in water. Values followed by different superscript letters are statistically different from each other, as determined by a post hoc Tukey HSD test with a *p*-value limit of 0.05 following one-way ANOVA, for which the *F* value (df = 2) is given with *p* values depicted as follows: • = ≤ 0.1, * = ≤ 0.05, ** = ≤ 0.01 *** = < 0.001.

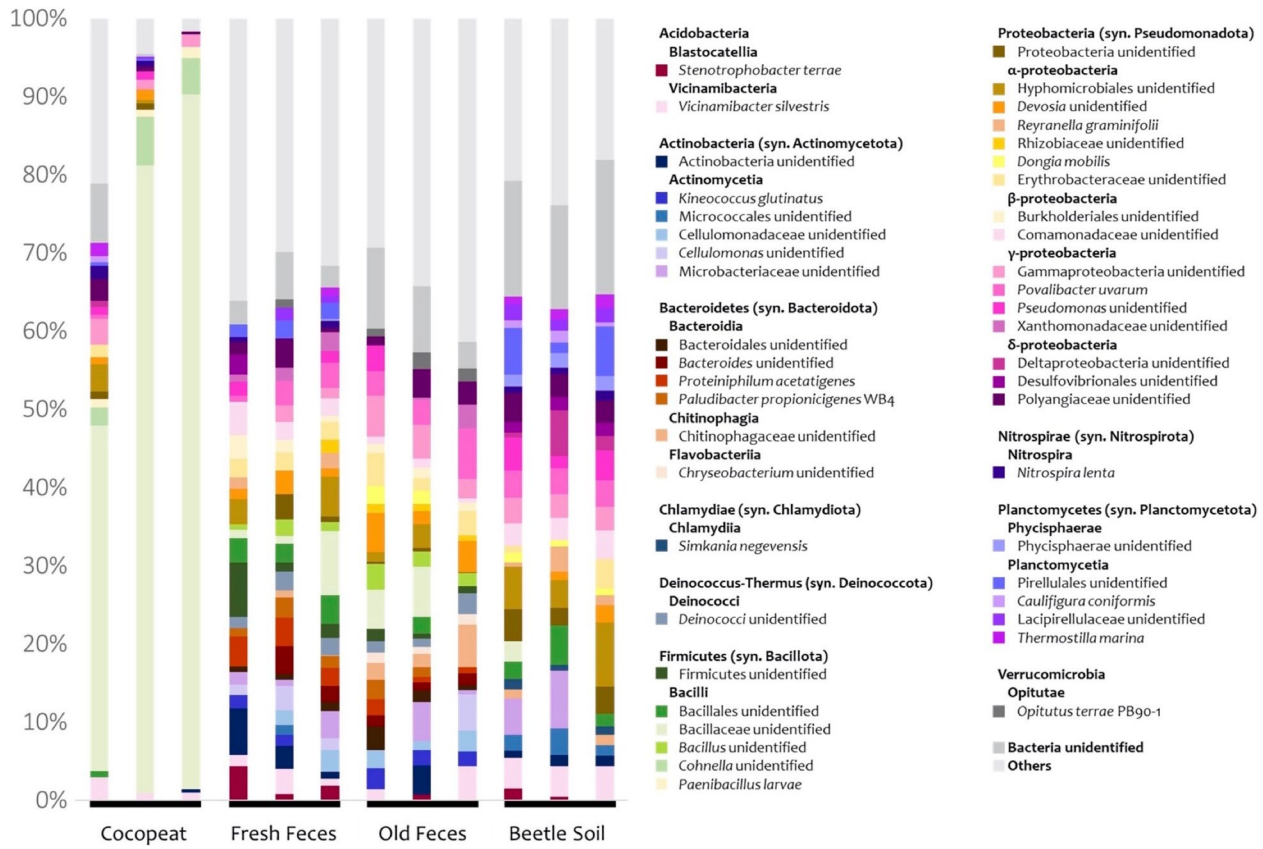


Fig. 1 Microbe relative abundance of *Oryctes rhinoceros* frass and diet by taxonomic composition

Table 3 Frequency of occurrence of each microbial clade among cocopeat, fresh frass, old frass, and beetle soil, with its potential functions

Assigned taxonomy	Potential functions associated with plants based on literature	Cocopeat	Fresh frass	Old frass	Beetle soil
Acidobacteriota (syn. Acidobacteria)					
Stenotrophobacter terrae	PGPR (Sato et al. 2023)	□	■	□	□
Vicinamibacter silvestris	Alleviating the accumulation of heavy metals in plants (Yu et al. 2023)	■	■	□	■
Actinomycetota (syn. Actinobacteria)					
Micrococcales unidentified	NA	□	□	□	■
Cellulomonadaceae unidentified	Cellulolytic/lignocellulytic (Yeager et al. 2017)	□	□	■	□
Microbacteriaceae unidentified	Cellulolytic (Bashir et al. 2013)	□	■	□	□
Cellulomonas unidentified	Cellulolytic/lignocellulytic (Yeager et al. 2017; Poulsen et al. 2016)	□	■	□	□
Kineococcus glutinatus	NA	□	□	■	□
Bacteroidota (syn. Bacteroidetes)					
Bacteroidales unidentified	Cellulolytic (Naas et al. 2015)	□	■	■	□
Chitinophagaceae unidentified	Denitrification (Ni et al. 2023), PGPR (Madhaiyan et al. 2015)	□	□	■	□
Bacteroides unidentified	Cellulolytic/xylanolytic (Daniel et al. 1995; Forsberg et al. 1981)	□	□	■	□
Chryseobacterium unidentified	Phosphate solubilization, PGPR (Singh et al. 2013)	□	□	■	□
Paludibacter propionicigenes WB4	Nitrogen fixation (Ceja-Navarro et al. 2014; Tai et al. 2016; Sapountzis et al. 2016)	□	■	□	□
Proteiniphilum acetatigenes	Cellulolytic, hemicellulolytic, ligninolytic (Thongbunrod and Chaiprasert 2023)	□	■	■	□
Chlamydiota (syn. Chlamydiae)					
Simkania negevensis	NA	□	□	□	■
Deinococcota (syn. Deinococcus-Thermus)					
Deinococci unidentified	Alleviating the accumulation of heavy metals in plants (Dai et al. 2021)	□	■	■	□
Bacillota (syn. Firmicutes)					
Bacillales unidentified	PGPR, nitrogen fixation	□	■	□	■
Bacillaceae unidentified	PGPR, nitrogen fixation (Mandic-Mulec et al. 2015)	■	■	□	□
Bacillus unidentified	PGPR (Sansinenea 2019), nitrogen fixation (Mandic-Mulec et al. 2015)	□	■	■	□
Cohnella unidentified	Xylanolytic (Hameed et al. 2013; Pisa et al. 2022)	■	□	□	□
Paenibacillus larvae	PGPR, nitrogen fixation, phosphate solubilization, production of IAA, release of siderophores (Grady et al. 2016)	■	□	□	□
Pseudomonadota (syn. Proteobacteria)					
Burkholderiales unidentified	PGPR (Kurepin et al. 2014)	□	■	■	□
Desulfovibrionales unidentified	Nitrogen fixation (Sayavedra et al. 2021)	□	□	□	■
Gammaproteobacteria unidentified	PGPR (Kumar Ghosh et al. 2015; Kuan et al. 2016)	■	□	■	■
Deltaproteobacteria unidentified	Nitrogen fixation (Langwig et al. 2022)	□	□	□	■
Hyphomicrobiales unidentified	Nitrogen fixation (Im et al. 2006; Wiegel et al. 1978)	□	□	□	■
Comamonadaceae unidentified	Sulfur fertilization (Bao and Li 2017; Schmalenberger et al. 2008), nitrification and denitrification (Bao and Li 2017)	□	■	■	■
Erythrobacteraceae unidentified	NA	□	■	■	□
Polyangiaceae unidentified	NA	■	■	■	■
Rhizobiaceae unidentified	Nitrogen fixation (Lindstrom and Mousavi 2020)	□	□	■	□
Xanthomonadaceae unidentified	Pectinolytic (Vorhölter et al. 2012)	□	■	□	□
Devosia unidentified	Nitrogen fixation (Rivas et al. 2002), PGPR (Chhetri et al. 2023), Bioremediation (Talwar et al. 2020)	□	■	■	□
Dongia mobilis	NA	□	□	□	■
Povalibacter uvarum	NA	□	■	■	■

Table 3 (continued)

Assigned taxonomy	Potential functions associated with plants based on literature	Cocopeat	Fresh frass	Old frass	Beetle soil
Pseudomonas unidentified	PGPR (Vorhölter et al. 2012; Preston 2004; Mercado-Blanco and Bakker 2007)	□	□	□	■
Reyranella graminifolii	Nitrogen fixation (Li et al. 2022), PGPR (Arp et al. 2014; Zhang et al. 2023)	□	□	□	■
Nitrospirota (syn. Nitrospirae)					
Nitrospira lenta	Nitrification (Daims et al. 2015)	□	□	□	■
Planctomycetota (syn. Planctomycetes)					
Lacipirellulaceae unidentified	Xylanolytic and pectinolytic (Dedysh et al. 2020)	□	□	□	■
Phycisphaerae unidentified	Xylanolytic (Naumoff et al. 2022)	□	□	□	■
Pirellulales unidentified	Xylanolytic and pectinolytic (Dedysh et al. 2020)	□	■	□	■
Caulifigura coniformis	NA	□	□	□	■
Thermostilla marina	NA	□	□	□	■
Verrucomicrobiota (syn. Verrucomicrobia)					
Opitutus terrae PB90-1	PGPR, phosphate solubilization, production of IAA, release of siderophores (Bünger et al. 2020)	□	□	■	□

IAA Indole-3-acetic acid; *NA* not applicable; *PGPR* plant growth promoting (rhizo)bacteria

Microbiome analysis

The 16S V3 and V4 sequences representing microbiomes from the food sources and frass contained 49,550 high-quality and filtered sequences, and produced 308 amplicon sequence variants (ASVs) with a median of 4272 reads per sample and 66 ASVs per sample. A total of one kingdom (Bacteria), 13 phyla, 27 classes, 39 orders, 58 families, 61 genera, and 41 species were classified from the total ASVs. The phyla Firmicutes (syn. Bacillota), Proteobacteria (syn. Pseudomonadota), Actinobacteria (syn. Actinomycetota), Bacteroidetes (syn. Bacteroidota), and Planctomycetes (syn. Planctomycetota), were the five most abundant (Fig. 1). There was only one 100% shared microbe among every sample, identified as a member of Polyangiaceae (Deltaproteobacteria, syn. Myxococcota) (Fig. 1 & Table 3).

The microbial species diversity of the commercial cocopeat was significantly lower than old or new frass and the lived-in substrate ["beetle soil"] (Fig. 2). The cocopeat microbiome was predominantly dominated by Firmicutes, especially unidentified Bacillaceae, a family which includes many plant-growth-promoting-bacteria (rhizobacteria) and nitrogen fixers (Fig. 1 & Table 3). By contrast, there was no specific group dominant among frass or beetle soil samples (Fig. 1), but two microbes, an unidentified Comamonadaceae and *Povalibacter uvarum* (Steroidobacterales: Steroidobacteraceae), were found in all frass and beetle soil samples (Fig. 1 & Table 3). The frass and beetle soil exhibited similarly high species diversity but distinct microbiomes, containing many species PICRUSt2 analysis suggested are potentially beneficial for plants (Figs. 1, 3 & Table 3). The permanova test indicated significant differences ($p < 0.0001$) in microbial structures among cocopeat, beetle soil, fresh frass, and old frass. However, as the pairwise Adonis did not reveal any significant differences between groups ($p > 0.05$), uncertainty persists regarding which group differs significantly from the others.

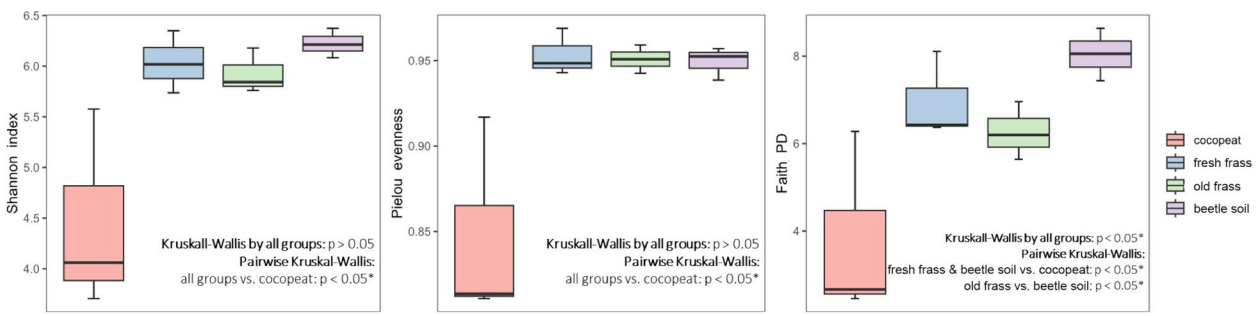


Fig. 2 Alpha diversity indices of the microbiomes

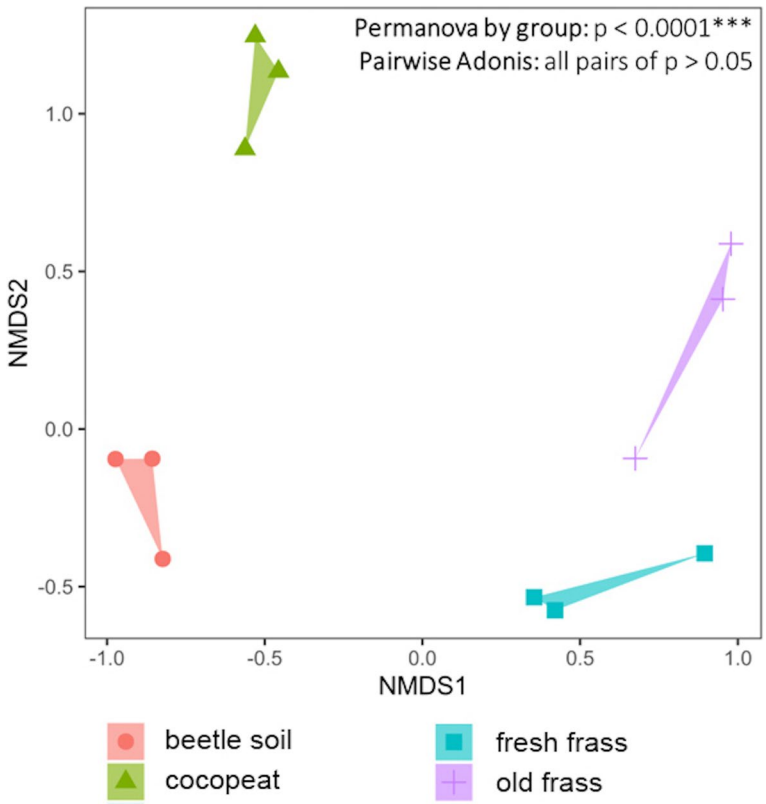


Fig. 3 Non-metric multidimensional scaling (nMDS) plot of the microbiomes (stress = 0.109). The microbial structure is depicted using the Bray–Curtis dissimilarity matrix

Figure 4 visually depicts comparative functional potentials along with corresponding statistical analyses for the different microbiomes' predicted capabilities of phytohormone synthesis, nitrogen fixation, phosphorus solubilization, sulfur oxidation, and depolymerization of plant cell walls and exoskeletons. Significant differences ($p < 0.05$) were observed in the functions of phytohormone synthesis (auxins and cytokinins), nitrogen fixation, phosphorus solubilization, sulfur oxidation, and hemicellulose degradation between groups. However, as the pairwise Wilcox test did not reveal any significant differences between groups ($p > 0.05$), uncertainty persists regarding which group differs significantly from the others. In addition to the statistical results, visually, the functions of phytohormone synthesis, nitrogen fixation, phosphorus solubilization, and sulfur oxidation showed a lower relative abundance in cocopeat compared to others. Synthesis of all depolymerizers, pectinases, and chitinases was more relatively abundant in the frass (especially old frass) microbiomes than the cocopeat or beetle soil (Fig. 4). The cocopeat microbiome displayed a greater relative abundance of hemicellulase synthesis than the beetle soil.

Plant growth tests

The effect of the frass on growth of *Arabidopsis thaliana* plants was evaluated (Fig. 5a,b). The plants growing on potting soil containing frass were noticeably and statistically larger (ANOVA: $F_2 = 8.826$, $p < 0.001$). In the case of the pots containing 40% frass, the biomass of the plants was significantly ($p < 0.05$) higher than in plants containing only the potting mix by 0.82 g per plant, representing an increase of 27% of their biomass (Table 4). There was no significant difference in dry weight or water content between treatments ($p < 0.05$). The frass-amended plants also started flowering earlier than plants growing on the control soil, and the latter were the last ones to start bolting. This was illustrated by the enhanced height of the inflorescence of plants grown on 40% frass at the time of harvest (30 days after planting) (ANOVA: $F_2 = 7.545$, $p < 0.01$). On average, these plants were 6.7 cm taller than those grown on control soil (Table 4). Therefore, the frass had a positive effect on the growth of the plants and led to plants that grew bigger and faster than plants grown on control soil.

With and without mixing, the pH of the potting soil and frass mixture was 5.7, suggesting the potting soil is well buffered and the frass does not have a strong buffering

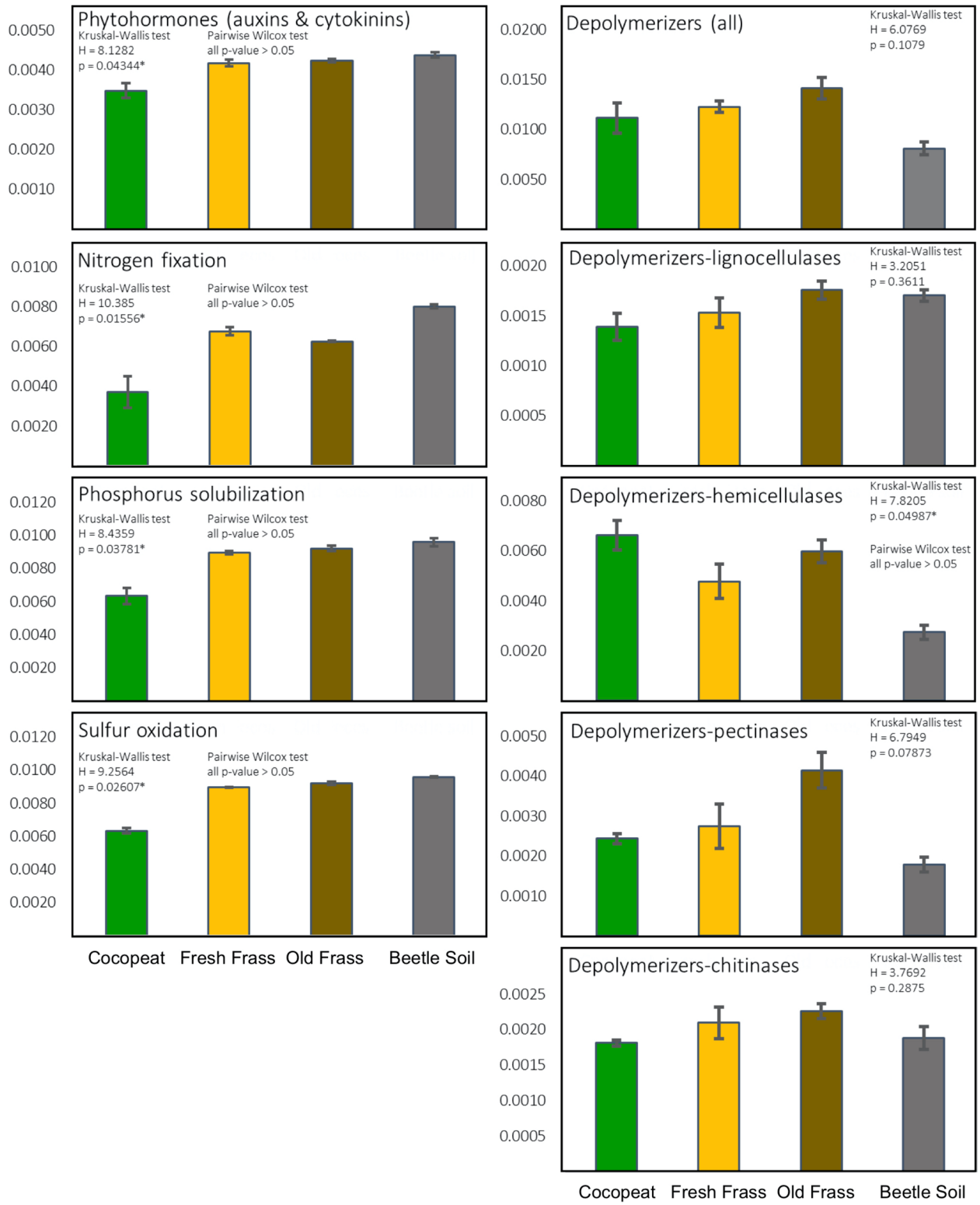


Fig. 4 Relative abundance of the functional potentials from the microbiome. The functions were predicted using PICRUSt2, focusing on genes and enzymes related to the provision of phytohormone synthesis, nitrogen fixation, phosphorus solubilization, sulfur oxidation, plant cell wall degradation, and exoskeleton degradation

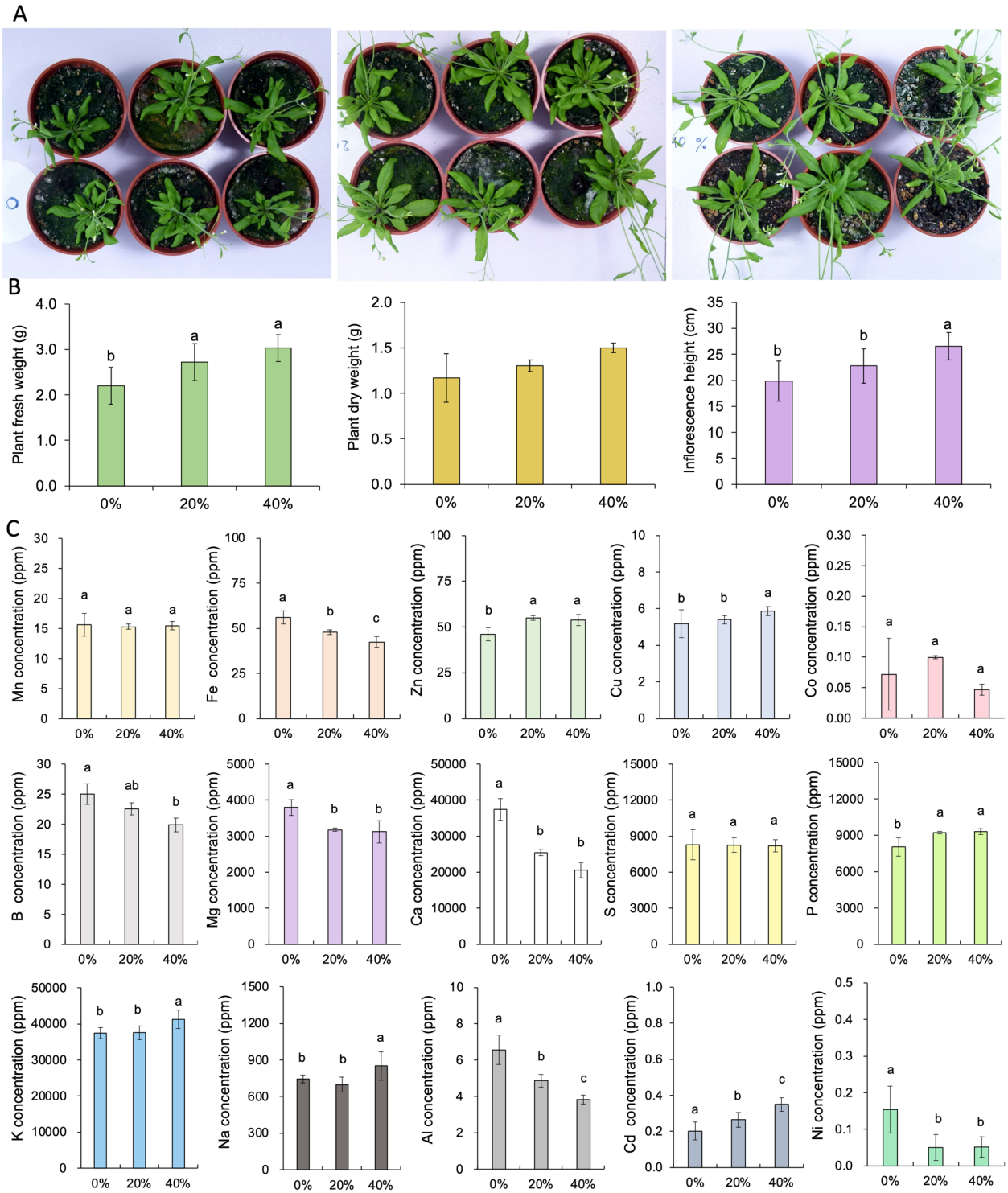


Fig. 5 Effect of fertilization with the beetle frass on the growth of *Arabidopsis thaliana*. **A** Pictures of a representative replicate of the experiment depicting 0, 20, and 40% frass supplementation of the soil. **B** Enhanced growth of plants grown on frass-containing soil. Fresh weight (left) and dry weight (middle) of the plants and height of the inflorescence at 7 weeks (right). **C** Elemental profile of the plants established by ICP-MS. Bars with different letters are statistically significant at $p < 0.05$ using a Duncan test

Table 4 Means ± Standard deviations for plant growth parameters for *Arabidopsis thaliana* grown in potting soil supplemented with 0, 20, or 40% *Oryctes rhinoceros* frass

% frass	Plant fresh weight (g)	Plant dry weight (g)	Water content (%)	Inflorescence height (cm after 30 days)
0%	2.2026 ± 0.4069[b]	1.1683 ± 0.2684	90.8641 ± 1.2059	19.8611 ± 3.8575[b]
20%	2.7191 ± 0.4088[a]	1.3040 ± 0.0626	91.0047 ± 0.2827	22.7625 ± 3.2826[b]
40%	3.0290 ± 0.2944[a]	1.4986 ± 0.0523	91.2163 ± 0.6709	26.5647 ± 2.6220[a]
$F_{(2)}$ p	8.826***			7.545**

Values followed by different superscript letters are statistically different from each other, as determined by a Duncan test with a p-value limit of 0.05 following one-way ANOVA, for which the F value (df = 2) is given with p values depicted as follows: • = ≤ 0.1, * = ≤ 0.05, ** = ≤ 0.01 *** = < 0.001.

capacity, and excluding any effect of pH on growth. To evaluate the impact of the frass on the nutritional status of the plants, the concentration of several essential nutrients and some non-essential elements in the harvested plants were determined by ICP-MS (Fig. 5c, Supplementary Table 1). Plants grown on frass-supplemented soil had significantly increased zinc (Zn), copper (Cu), phosphorus (P), potassium (K), sodium (Na), and cadmium (Cd) concentrations. In contrast, their concentrations in iron (Fe), boron (B), magnesium (Mg), calcium (Ca), aluminum (Al), and nickel (Ni) were lower than in control plants. The most notable changes in terms of amplitude were the Ca, Al, and Ni concentrations which decreased by 45%, 42%, and 70% respectively.

Table 5 Means ± Standard deviations for elemental concentrations (mg/kg) in *Oryctes rhinoceros* frass and potting soil used in plant growth tests

Elements	Frass	Soil	t & p
Al	2022 ± 247	68 ± 6.3	− 11.18**
B	70.9 ± 14.4	43.8 ± 5.9	− 2.466•
Ca	56,619 ± 19,292	5613 ± 523	− 3.738*
Cr	5.4 ± 0.4	1.59 ± 0.4	− 8.914**
Cu	32.4 ± 3.2	36.3 ± 5.8	− 0.844
K	14,937 ± 173.4	1048 ± 420.2	− 43.21***
Mg	11,969 ± 3330	2278 ± 249	− 4.104*
Mn	261 ± 23.5	< LOD	
Na	1562 ± 86.4	287 ± 41.2	− 18.83**
P	22,667 ± 2175.8	9619 ± 331	− 8.384**
As	< LOD	< LOD	
Cd	26.31 ± 9.49	30.85 ± 5.31	− 0.723
Fe	3195 ± 65.1	4639 ± 49.9	− 30.48***
Hg	< LOD	< LOD	
Ni	< LOD	< LOD	
Pb	4.470 ± 0.56	4.617 ± 1.0	− 0.219
Rb	25.050 ± 0.78	10.767 ± 0.54	− 26.118***
S	1573 ± 10.1	4794 ± 51.7	− 106.018***
Si	< LOD	6481 ± 225.0	
Sr	126.0 ± 0.687	33.96 ± 0.814	− 149.724***
Ti	< LOD	< LOD	
Zn	96.03 ± 2.4	22.18 ± 0.16	− 52.188***
Zr	25.92 ± 5.4	19.25 ± 1.16	− 2.093•

Concentrations of the first 10 elements listed were measured via ICP-OES, the rest with pXRF. Means were compared with unpaired t-tests and the p values are depicted as follows: • = ≤ 0.1, * = ≤ 0.05, ** = ≤ 0.01 *** = < 0.001. LOD = limit of detection

Table 5 compares the elemental concentrations of the frass and the initial potting soil determined by ICP-OES. The frass had significantly higher ($p < 0.05$) concentrations of most elements, in particular aluminum, potassium, and calcium with about an order of magnitude difference. Potting soil had greater concentrations of cadmium, iron, sulfur (S), and silicon (Si).

Discussion

One goal of composting is to lower the C:N ratio of raw materials (commonly ≥ 40) to a level closer to that of healthy soil (15–25). As the *O. rhinoceros* frass C:N ratio (22.8 ± 2.0) is within this range [and comparable to the previously reported C:N value of 14.6 ± 0.85 (Beesigamukama et al. 2022)], the frass can potentially be used directly as a soil amendment without any further composting. This was supported by the *Arabidopsis* experiments that showed higher growth with greater frass content in the soil, and the lack of evidence of phytotoxicity. Based on these results, the frass matches the definition of a biostimulant, enhancing plant growth without increasing its nutrient acquisition. It also seems more useful as a growth substrate itself, similar to how cocopeat can be used. Preliminary studies confirm that plants can germinate and grow in 100% frass, but robust experimentation would be needed to check how effective *O. rhinoceros* frass is as a growth substrate. Unknown factors include water retention and drainage in the frass that could cause nutrient leakage.

The frass microbiomes included several microbes with potential for phytohormone (auxins and cytokinins) production, nitrogen fixation, phosphorus solubilization, sulfur oxidation, and depolymerization of woody materials and chitins (Table 3 & Fig. 4). These characteristics are known to enhance plant growth and development rate (Egamberdieva et al. 2017; Richardson et al. 2009; Ranadev et al. 2023; Xu et al. 2018). In Fig. 4, the relative abundances of functional potentials from the microbiome related to phytohormones, nitrogen fixation, phosphorus solubilization, and sulfur oxidation vary significantly across different groups (p-value < 0.05), exhibiting distinct visual differences between cocopeat and frass. However, detailed differences between groups remain uncertain due to the insignificance of the pairwise Wilcoxon tests (p-value > 0.05). Possible reasons for this could include the limited sample size, which might not be large enough to detect small differences statistically, and the variability of individual microbiomes, making it challenging to distinguish true differences from background noise. Within the commonly found microbes in all frass samples as shown in Table 3 are clades with known cellulolytic, hemicellulolytic, ligninolytic, nitrogen fixation, bioremediation, or sulfur fertilization potential. These may assist in plant growth and development, thus reflecting the observed, positive effect on the growth of *Arabidopsis thaliana*.

The presence of chitinolytic microbes in the frass may help protect the plants from pathogenic fungi and nematodes while releasing nutrients from chitin that promote plant growth (Debode et al. 2016; Kisaakye et al. 2024; Shobade et al. 2024). Direct chitin supplementation of soil is known to increase both the abundance of naturally occurring chitinolytic microbes and total chitinase activity, with subsequent benefits in plant growth (Poulsen et al. 2008; Fan et al. 2022). Insect frass itself likely contains some chitin from the peritrophic membrane secreted to surround the food bolus (Chiang and Shelomi 2023). As a potential source of both chitin and chitinolytic microbes, frass could provide greater benefits than amending soil with one or the other.

Plant pathogenic microbes were not observed in this study, nor are *O. rhinoceros* known vectors of any plant pathogen to date. *O. rhinoceros* are capable of vectoring insect pathogens, however: specifically their own pathogenic bacteria, *Acinetobacter calcoaceticus* (Pseudomonadales: Moraxellaceae) (Kannan et al. 1980); pathogenic nudivirus, OrNv (Huger 2005); and their possibly pathogenic picorna-like virus, OrPV (Etebari et al. 2020). Overall, the beetle frass seems to carry beneficial bacteria while posing low risk to crops, and its formulation as a soil amendment should ideally preserve this microbiome by eschewing or minimizing pre-sterilization. Not using the frass on crops susceptible to the same bacterial infections as the palms (Arecaecae) that *O. rhinoceros* preferentially feed on would minimize any risk from transmitted pathogens. Alternatively, the ability of the beetle frass to transmit pest management viruses could mean the frass can be formulated as a combined soil amendment and biocontrol virus delivery tool, pending dedicated research on this possibility.

As an aside, the plant cell wall degrading enzymes in the microbiome likely assist the insect in fully digesting wood (Shelomi et al. 2019), and the particle size data confirms that the ingested cocopeat is significantly digested in the larval gut. Compost particle size correlates with maturity and quality (Mishra and Yadav 2022) and can affect oxygen uptake and disease suppression (Lozano et al. 2009), though the significance of the current data for the frass particles regarding compost quality is not clear.

The observed benefit of the frass to the plants is not necessarily due to its nutritional content alone. While frass was nutrient dense, the control potting soil is also nutrient-rich (Table 5) and already constitutes an optimal growth medium that does not require additional nutrients, nor did all nutrients increase in concentration in the plants when soil was replaced with frass (Fig. 5c, Supplementary Table 1). The observed changes in element concentrations in the plants were overall moderate, and some elements in plants reared in the potting soil with beetle frass supplementation showed reductions compared to the control group, such as Ca, Al, and Ni. This is striking given that the Al and Ca concentrations in the frass were over an order of magnitude greater than in the soil (Table 5). The Ca concentration in the plants grown with frass group was still within the optimal range for plant growth (Marschner 1995). As for Al and Ni, higher concentrations of them are known to inhibit and disturb the plant's growth (Singh et al. 2017; Mustafa et al. 2023). Overall, the element concentrations measured in the soil where plants were grown suggest that supplementation with frass significantly increased most minerals in the plant, correlating with but not necessarily or solely causing overall positive effects on the plants. We hypothesize that sterilized frass lacking in beneficial microbes but retaining the same nutrients may not produce the same results as control frass.

Before now, no study had thoroughly investigated the potential of scarab beetles, especially coconut rhinoceros beetles (*O. rhinoceros*), as a plant fertilizer

by combining nutritional analyses with microbiome data and plant growth assays to provide a more solid and comprehensive understanding of its applicational potential. While one previous study compared the nutritional properties of frass from *Oryctes rhinoceros* with eight other, common edible insects (Beesigamukama et al. 2022), it did not consider the effect of microbial assistance in relation to plant growth and development, nor did it conduct plant growth assays beyond germination tests. Note that this previous study on *O. rhinoceros* frass found moderate phytotoxicity of the frass as measured by reduced radical elongation in cabbage seeds reared on filter paper moistened with 10% frass compared to those on distilled water; however, the germination rate for the *O. rhinoceros* frass seeds was 93.3 ± 6.7%, equivalent to the data for black soldier fly in that study that was identified as non-phytotoxic. The current study found no evidence of phytotoxicity: by contrast, plants reared on soil amended 40% with frass outperformed soil without frass. However, Na levels were higher in such plants than the control (Fig. 5c), and sodium concentrations in the frass were significantly higher ($p < 0.01$, Table 5) than the soil, suggesting the frass may contain unideal levels of salt that may need to be mitigated or kept in mind when determining ideal usage parameters for the frass.

In conclusion, this study demonstrated beneficial impacts on plant growth and development from direct use of *O. rhinoceros* frass as a soil amendment, suggesting potential applications in agriculture as a biostimulant or growth substrate. The beetle can be said to have biocomposted the ingested cocopeat into a more valuable substance (frass), akin to other forms of composting, and this frass is a mature compost that can be used directly as a soil amendment. Use of beetle frass as soil amendment could provide additional income opportunities for beetle rearers, particularly those raising edible species. Valorizing the waste of insects into sustainable soil amendments can be both profitable and environmentally helpful as a replacement for fertilizers, a tool in permaculture or hydroponics, or restoration of degraded soils (Regina and Daniel 2021). As a side note, the authors observed that fungi could grow on beetle frass after a while if it was improperly stored. This suggests that the frass could be a potential mushroom-growing substrate once sterilized and hydrated through pasteurization.

Abbreviations

ASV	Amplicon sequence variant
nMDS	Non-metric multidimensional scaling
PGPR	Plant growth promoting (rhizo)bacteria

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40529-025-00459-x.

Additional file 1.

Additional file 2: Figure 1. Particle size distribution for undigested, commercial cocopeat (a) and *Oryctes rhinoceros* frass (b). Sync analysis type: Diff/Im g. Particle size classes are listed as volumetrically averaged diameters. The inserts show the span of particle size classes of the cocopeat feed and beetle frass. Each distribution (D#) value is the maximum size, in µm, for the #% smallest particles. Kg = Kurtois value. Mz = graphic mean. SD = standard deviation. Ski = Inclusive Graphic Skewness.

Acknowledgements

The authors would like to thank Chih-Hsin Cheng and Ting-Feng Yeh for their contributions to earlier drafts of this manuscript, Min-Rou Chiang for beetle rearing, Ching-Yuan Chang for preparing the plant samples for ICP-MS, and Kuo-Chen Yeh and I-Chien Tang from the Agricultural Biotechnology Research Center of Academia Sinica who performed the ICP-MS measurements.

Author contributions

CJH reared the insects, performed the microbiome and functional profile analyses, and wrote part of the manuscript. ZYH and PHW performed chemical soil analysis. LG performed the plant growth studies and ICP-MS. CHK performed the fiber analysis and wrote part of the manuscript. MS conceived of the study, provided biological materials, performed microbiological analysis, and wrote part of the manuscript.

Funding

This work was supported by the Ministry of Education of Taiwan Grants 111L7822, 112L7814, and 113L7801, and the National Science and Technology Council of Taiwan Grants MOST-109-2311-B-002-016-MY3, NSTC-113-2628-B-002-015-MY3, and NSTC-114-2311-B-002-017.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available in the NCBI GenBank depository (Bioproject: PRJNA1052367). The data corresponding with Fig. 5c is available as Supplementary Table 1.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 22 September 2024 Accepted: 28 March 2025
Published online: 05 August 2025

References

Arp A, Munyaneza JE, Crosslin JM, Trumble J, Bextine B (2014) A global comparison of *Bactericera cockerelli* (Hemiptera: Triozidae) microbial communities. Environ Entomol 43:344–352. https://doi.org/10.1603/EN13256

Bao P, Li GX (2017) Sulfur-driven iron reduction coupled to anaerobic ammonium oxidation. Environ Sci Technol 51:6691–6698. https://doi.org/10.1021/acs.est.6b05971

Barragán-Fonseca KY, Nurfikari A, van de Zande EM, Wantulla M, van Loon JJA, de Boer W, Dicke M (2022) Insect frass and exuviae to promote plant growth and health. Trends Plant Sci 27:646–654. https://doi.org/10.1016/j.tplants.2022.01.007

Bashir Z, Kondapalli VK, Adlakha N, Sharma A, Bhatnagar RK, Chandel G, Yazdani SS (2013) Diversity and functional significance of cellulolytic microbes living in termite, pill-bug and stem-borer guts. Sci Rep 3:2558. https://doi.org/10.1038/srep02558
Beesigamukama D, Subramanian S, Tanga CM (2022) Nutrient quality and maturity status of frass fertilizer from nine edible insects. Sci Rep 12:7182. https://doi.org/10.1038/s41598-022-11336-z
Blakstad JI, Strimbeck R, Poveda J, Bones AM, Kissen R (2023) Frass from yellow mealworm (*Tenebrio molitor*) as plant fertilizer and defense priming agent. Biocatal Agric Biotechnol. https://doi.org/10.1016/j.bcab.2023.102862
Bolaji O, Bamidele D, Adeboye A, Tanimola A (2021) Influence of preparation and processing methods on the physico-chemical properties of *Oryctes rhinoceros* larvae. Croatian J Food Sci Technol 13:148–154. https://doi.org/10.17508/CJFST.2021.13.2.02
Bolyen E, Rideout JR, Dillon MR et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9
Bremner J (1960) Determination of nitrogen in soil by the Kjeldahl method. J Agric Sci 55:11–33. https://doi.org/10.1017/S0021859600021572
Bünger W, Jiang X, Muller J, Hurek T, Reinhold-Hurek B (2020) Novel cultivated endophytic verrucomicrobia reveal deep-rooting traits of bacteria to associate with plants. Sci Rep 10:8692. https://doi.org/10.1038/s41598-020-65277-6
Ceja-Navarro JA, Nguyen NH, Karaoz U et al (2014) Compartmentalized microbial composition, oxygen gradients and nitrogen fixation in the gut of *Odontotaenius disjunctus*. ISME J 8:6–18. https://doi.org/10.1038/ismej.2013.134
Chhetri G, Kim I, Seo T (2023) Devosia oryzisoli sp. nov., a novel moderately halotolerant bacterium isolated from the roots of rice plants and genome mining revealed the biosynthesis potential as plant growth promoter. Antonie Van Leeuwenhoek 116:231–242. https://doi.org/10.1007/s10482-022-01800-1
Chiang M-R, Shelomi M (2023) Anatomical changes of the beetle digestive tract during metamorphosis correspond to dietary changes. J Morphol 284:e21575. https://doi.org/10.1002/jmor.21575
Cito A, Longo S, Mazza G, Dreassi E, Francardi V (2017) Chemical evaluation of the *Rhynchophorus ferrugineus* larvae fed on different substrates as human food source. Food Sci Technol Int 23:529–539. https://doi.org/10.1177/1082013217705718
Dahl C (2015) Cytoplasmic sulfur trafficking in sulfur-oxidizing prokaryotes. IUBMB Life 67:268–274. https://doi.org/10.1002/iub.1371
Dai Z, Guo X, Yin H, Liang Y, Cong J, Liu X (2014) Identification of nitrogen-fixing genes and gene clusters from metagenomic library of acid mine drainage. PLoS ONE 9:e87976. https://doi.org/10.1371/journal.pone.0087976
Dai S, Chen Q, Jiang M et al (2021) Colonized extremophile Deinococcus radiodurans alleviates toxicity of cadmium and lead by suppressing heavy metal accumulation and improving antioxidant system in rice. Environ Pollut 284:117127. https://doi.org/10.1016/j.envpol.2021.117127
Daims H, Lebedeva EV, Pjevac P et al (2015) Complete nitrification by Nitrospira bacteria. Nature 528:504–509. https://doi.org/10.1038/nature16461
Daniel AS, Martin J, Vanat I, Whitehead TR, Flint HJ (1995) Expression of a cloned cellulase/xylanase gene from Prevotella ruminicola in Bacteroides vulgatus, Bacteroides uniformis and Prevotella ruminicola. J Appl Bacteriol 79:417–424. https://doi.org/10.1111/j.1365-2672.1995.tb03156.x
Davidowitz G (2021) Habitat-centric versus species-centric approaches to edible insects for food and feed. Curr Opin Insect Sci 48:37–43. https://doi.org/10.1016/j.cois.2021.09.006
de Mendiburu F, Yaseen M (2020) agricolae: statistical procedures for agricultural research. version 1.4.0 edn. R package,
Debode J, De Tender C, Soltaninejad S, Van Malderghem C, Haegeman A, Van der Linden I, Cottyn B, Heyndrickx M, Maes M (2016) Chitin mixed in potting soil alters lettuce growth, the survival of zoonotic bacteria on the leaves and associated rhizosphere microbiology. Front Microbiol 7:565. https://doi.org/10.3389/fmicb.2016.00565
Dedysh SN, Kulichevskaya IS, Beletsky AV, Ivanova AA, Rijpstra WIC, Damste JSS, Mardanov AV, Ravin NV (2020) *Lacipirellula parvula* gen. nov., sp. nov., representing a lineage of planctomycetes widespread in low-oxygen habitats, description of the family *Lacipirellulaceae* fam. nov. and proposal of the orders *Pirellulales* ord. nov., *Gemmatales* ord. nov. and *Isosphaerales* ord. nov. Syst Appl Microbiol 43:126050. https://doi.org/10.1016/j.syapm.2019.126050
Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631. https://doi.org/10.1038/nrmicro954
Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38:669–688. https://doi.org/10.1038/s41587-020-0548-6
Egamberdieva D, Wirth SJ, Alqarawi AA, Abd Allah EF, Hashem A (2017) Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Front Microbiol 8:2104. https://doi.org/10.3389/fmicb.2017.02104
Estaki M, Jiang L, Bokulich NA et al (2020) QIIME 2 enables comprehensive end-to-end analysis of diverse microbiome data and comparative studies with publicly available data. Curr Protoc Bioinformatics 70:e100. https://doi.org/10.1002/cpbi.100
Etebari K, Shelomi M, Furlong MJ (2020) Identification of a novel Picorna-like virus in coconut rhinoceros beetles (*Oryctes rhinoceros*). Virus Res 287:198100. https://doi.org/10.1101/2020.04.15.042309
Fan Y, Liu J, Liu Z et al (2022) Chitin amendments eliminate the negative impacts of continuous cropping obstacles on soil properties and microbial assemblage. Front Plant Sci 13:1067618. https://doi.org/10.3389/fpls.2022.1067618
Forsberg CW, Beveridge TJ, Hellstrom A (1981) Cellulase and xylanase release from bacteroides succinogenes and its importance in the rumen environment. Appl Environ Microbiol 42:886–896
Frébort I, Kowalska M, Hluska T, Frebortova J, Galuszka P (2011) Evolution of cytokinin biosynthesis and degradation. J Exp Bot 62:2431–2452. https://doi.org/10.1093/jxb/err004
Frébortová J, Frebort I (2021) Biochemical and structural aspects of cytokinin biosynthesis and degradation in bacteria. Microorganisms. https://doi.org/10.3390/microorganisms9061314
Friedrich CG, Bardischewsky F, Rother D, Quentmeier A, Fischer J (2005) Prokaryotic sulfur oxidation. Curr Opin Microbiol 8:253–259. https://doi.org/10.1016/j.mib.2005.04.005
Gherlenda AN, Crous KY, Moore BD, Haigh AM, Johnson SN, Riegler M (2016) Precipitation, not CO2 enrichment, drives insect herbivore frass deposition and subsequent nutrient dynamics in a mature *Eucalyptus* woodland. Plant Soil 399:29–39. https://doi.org/10.1007/s11104-015-2683-2
Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC (2016) Current knowledge and perspectives of Paenibacillus: a review. Microb Cell Fact 15:203. https://doi.org/10.1186/s12934-016-0603-7
Hameed A, Hung MH, Lin SY et al (2013) Cohnella formosensis sp. nov., a xylanolytic bacterium isolated from the rhizosphere of *Medicago sativa* L. Int J Syst Evol Microbiol 63:2806–2812. https://doi.org/10.1099/ijs.0.045831-0
Han C-J, Cheng C-H, Yeh T-F, Pauchet Y, Shelomi M (2024) Coconut rhinoceros beetle digestive symbiosis with potential plant cell wall degrading microbes. Npj Biofilms Microbiomes 10:34. https://doi.org/10.1038/s41522-024-00505-9
Holzinger A, Hink L, Sehl E, Rüppel N, Lehndorff E, Weig AR, Agarwal S, Horn MA, Feldhaar H (2023) Biodegradable polymers boost reproduction in the earthworm *Eisenia fetida*. Sci Total Environ 892:164670. https://doi.org/10.1016/j.scitotenv.2023.164670
Houben D, Daoulas G, Faucon M-P, Dulaurent A-M (2020) Potential use of mealworm frass as a fertilizer: impact on crop growth and soil properties. Sci Rep 10:4659. https://doi.org/10.1038/s41598-020-61765-x
Huger AM (2005) The Oryctes virus: its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). J Invertebr Pathol 89:78–84. https://doi.org/10.1016/j.jip.2005.02.010
Im WT, Kim SH, Kim MK, Ten LN, Lee ST (2006) *Pleomorphomonas koreensis* sp. nov., a nitrogen-fixing species in the order Rhizobiales. Int J Syst Evol Microbiol 56:1663–1666. https://doi.org/10.1099/ijs.0.63499-0
Kannan N, Shanmugasundaram S, Lakshmanan M (1980) Isolation of a bacterial pathogen from the coconut pest *Oryctes rhinoceros* L. Entomon 5:285–289
Keswani C, Singh SP, Cueto L et al (2020) Auxins of microbial origin and their use in agriculture. Appl Microbiol Biotechnol 104:8549–8565. https://doi.org/10.1007/s00253-020-10890-8

Kisaakye J, Beesigamukama D, Haukeland S, Subramanian S, Thiongo PK, Kelemu S, Tanga CM (2024) Chitin-enriched insect frass fertilizer as a biorational alternative for root-knot nematode (*Meloidogyne incognita*) management. Front Plant Sci. https://doi.org/10.3389/fpls.2024.1361739

Kuan KB, Othman R, Abdul Rahim K, Shamsuddin ZH (2016) Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. PLoS ONE 11:e0152478. https://doi.org/10.1371/journal.pone.0152478

Kumar Ghosh P, Kumar Sen S, Kanti Maiti T (2015) Production and metabolism of IAA by Enterobacter spp. (Gammaproteobacteria) isolated from root nodules of a legume *Abrus precatorius* L. Biocatal Agric Biotechnol 4:296–303. https://doi.org/10.1016/j.bcab.2015.04.002

Kurepin LV, Park JM, Lazarovits G, Bernards MA (2014) Burkholderia phytofirmans-induced shoot and root growth promotion is associated with endogenous changes in plant growth hormone levels. Plant Growth Regul 75:199–207. https://doi.org/10.1007/s10725-014-9944-6

Langwig MV, De Anda V, Dombrowski N, Seitz KW, Rambo IM, Greening C, Teske AP, Baker BJ (2022) Large-scale protein level comparison of deltaproteobacteria reveals cohesive metabolic groups. ISME J 16:307–320. https://doi.org/10.1038/s41396-021-01057-y

Li Q, Wan F, Zhao M (2022) Distinct soil microbial communities under *Ageratina adenophora* invasions. Plant Biol (Stuttg) 24:430–439. https://doi.org/10.1111/plb.13387

Liang JL, Liu J, Jia P, Yang TT, Zeng QW, Zhang SC, Liao B, Shu WS, Li JT (2020) Novel phosphate-solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. ISME J 14:1600–1613. https://doi.org/10.1038/s41396-020-0632-4

Lindstrom K, Mousavi SA (2020) Effectiveness of nitrogen fixation in rhizobia. Microb Biotechnol 13:1314–1335. https://doi.org/10.1111/1751-7915.13517

Lopes IG, Yong JWH, Lalander C (2022) Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives. Waste Manag 142:65–76. https://doi.org/10.1016/j.wasman.2022.02.007

Lovett GM, Ruesink AE (1995) Carbon and nitrogen mineralization from decomposing gypsy moth frass. Oecologia 104:133–138

Lozano J, Blok WJ, Termorshuizen AJ (2009) Effect of compost particle size on suppression of plant diseases. Environ Eng Sci 26:601–607. https://doi.org/10.1089/ees.2008.0002

Madhaiyan M, Poonguzhali S, Senthilkumar M, Pragatheswari D, Lee JS, Lee KC (2015) Arachidicoccus rhizosphaerae gen. nov., sp. nov., a plant-growth-promoting bacterium in the family Chitinophagaceae isolated from rhizosphere soil. Int J Syst Evol Microbiol 65:578–586. https://doi.org/10.1099/ijs.0.069377-0

Mandic-Mulec I, Stefanic P, van Elsas JD (2015) Ecology of bacillaceae. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.TBS-0017-2013

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, New York

McLean EO (1982) Soil pH and lime requirement. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, Part 2. Agron. Monogr. 9, 2 edn. ASA-CSSA-SSSA, Madison, Wisconsin, pp. 199–224

Mercado-Blanco J, Bakker PA (2007) Interactions between plants and beneficial Pseudomonas spp.: exploiting bacterial traits for crop protection. Antonie Van Leeuwenhoek 92:367–389. https://doi.org/10.1007/s10482-007-9167-1

Mishra SK, Yadav KD (2022) Assessment of the effect of particle size and selected physico-chemical and biological parameters on the efficiency and quality of composting of garden waste. J Environ Chem Eng 10:107925. https://doi.org/10.1016/j.jece.2022.107925

Mustafa A, Zulfiqar U, Mumtaz MZ et al (2023) Nickel (Ni) phytotoxicity and detoxification mechanisms: a review. Chemosphere 328:138574. https://doi.org/10.1016/j.chemosphere.2023.138574

Naas AE, MacKenzie AK, Dalhus B, Eijsink VGH, Pope PB (2015) Structural features of a bacteroidetes-affiliated cellulase linked with a polysaccharide utilization locus. Sci Rep 5:11666. https://doi.org/10.1038/srep11666

Naumoff DG, Kulichevskaya IS, Dedysh SN (2022) Genetic determinants of xylan utilization in Humisphaera borealis M1803T, a planctomycete of the class phycisphaerae. Microbiology 91:249–258. https://doi.org/10.1134/s002626172230004x

Nelson DW, Sommers LE (1982) Total carbon, organic carbon, and organic matter. In: Page AL, Miller RH, Keeney DR (eds) Methods of Soil Analysis, Part 2. Agron. Monogr. 9, 2 edn. ASA-CSSA-SSSA, Madison, Wisconsin, pp. 539–577

Ni R, Wang Y, Lei Y, Song L (2023) Response of denitrification microbiome to the nitrogen flux in three Gorges reservoir (TGR) sediments during two seasonal water fluctuation events. Environ Res 237:117025. https://doi.org/10.1016/j.envres.2023.117025

O'Leary NA, Wright MW, Brister JR et al (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733-745. https://doi.org/10.1093/nar/gkv1189

Oksanen FJ (2017) Vegan: community ecology package. R package Version 24–3

Osimani A, Milanović V, Cardinali F et al (2018) The bacterial biota of laboratory-reared edible mealworms (*Tenebrio molitor* L.): from feed to frass. Int J Food Microbiol 272:49–60. https://doi.org/10.1016/j.ijfoodmicro.2018.03.001

Pisa JH, Hero JS, Romero HG, Martinez MA (2022) A genome-proteome-based approach for xylan degradation by Cohnella sp. AR92. Environ Microbiol Rep 14:755–765. https://doi.org/10.1111/1758-2229.13113

Poulsen PH, Møller J, Magid J (2008) Determination of a relationship between chitinase activity and microbial diversity in chitin amended compost. Bioresour Technol 99:4355–4359. https://doi.org/10.1016/j.biortech.2007.08.042

Poulsen HV, Willink FW, Ingvorsen K (2016) Aerobic and anaerobic cellulase production by Cellulomonas ud. Arch Microbiol 198:725–735. https://doi.org/10.1007/s00203-016-1230-8

Poveda J (2021) Insect frass in the development of sustainable agriculture. A review. Agron Sustain Dev 41:5. https://doi.org/10.1007/s13593-020-00656-x

Poveda J, Jiménez-Gómez A, Saati-Santamaría Z, Usategui-Martín R, Rivas R, García-Fraile P (2019) Mealworm frass as a potential biofertilizer and abiotic stress tolerance-inductor in plants. Appl Soil Ecol 142:110–122. https://doi.org/10.1016/j.apsoil.2019.04.016

Pradipta AP, Wagiman FX, Witjaksono (2020) The potency of collecting larvae of *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) in the oil palm plantation. AGRIVITA J Agric Sci 42:153–159. https://doi.org/10.17503/agrivita.v42i1.2489

Preston GM (2004) Plant perceptions of plant growth-promoting Pseudomonas. Philos Trans R Soc Lond B Biol Sci 359:907–918. https://doi.org/10.1098/rstb.2003.1384

Ranadev P, Revanna A, Bagyaraj DJ, Shinde AH (2023) Sulfur oxidizing bacteria in agro ecosystem and its role in plant productivity-a review. J Appl Microbiol. https://doi.org/10.1093/jambio/lxad161

Regina M, Daniel M (2021) The insects as a workforce for organic fertilizers production – Insect Frass. In: Metin T, Ertan Y (eds) New generation of organic fertilizers. IntechOpen, Rijeka. https://doi.org/10.5772/intechopen.100144

Richardson AE, Barea J-M, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339. https://doi.org/10.1007/s11104-009-9895-2

Rivas R, Velazquez E, Willems A, Vizcaino N, Subba-Rao NS, Mateos PF, Gillis M, Dazzo FB, Martinez-Molina E (2002) A new species of Devosia that forms a unique nitrogen-fixing root-nodule symbiosis with the aquatic legume *Neptunia natans* (L.f.) druce. Appl Environ Microbiol 68:5217–5222. https://doi.org/10.1128/AEM.68.11.5217-5222.2002

Robeson MS 2nd, O'Rourke DR, Kaehler BD, Ziemski M, Dillon MR, Foster JT, Bokulich NA (2021) RESCRIPt: Reproducible sequence taxonomy reference database management. PLoS Comput Biol 17:e1009581. https://doi.org/10.1371/journal.pcbi.1009581

Rodríguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21. https://doi.org/10.1007/s11104-006-9056-9

Sansinenea E (2019) Bacillus spp.: as plant growth-promoting bacteria. Secondary metabolites of plant growth promoting rhizomicroorganisms. Springer Singapore, Singapore, pp 225–237

Santos PCD, Fang Z, Mason SW, Setubal JC, Dixon R (2012) Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. BMC Genomics. https://doi.org/10.1186/1471-2164-13-162
Sapountzis P, de Verges J, Rousk K, Cilliers M, Vorster BJ, Poulsen M (2016) Potential for nitrogen fixation in the fungus-growing termite symbiosis. Front Microbiol 7:1993. https://doi.org/10.3389/fmicb.2016.01993
Sato Y, Miwa T, Inaba T et al (2023) Microbially produced fertilizer provides rhizobacteria to hydroponic tomato roots by forming beneficial biofilms. Appl Microbiol Biotechnol 107:7365–7374. https://doi.org/10.1007/s00253-023-12794-9
Sayavedra L, Li T, Bueno Batista M, Seah BKB, Booth C, Zhai Q, Chen W, Narbad A (2021) Desulfovibrio diazotrophicus sp. nov., a sulfate-reducing bacterium from the human gut capable of nitrogen fixation. Environ Microbiol 23:3164–3181. https://doi.org/10.1111/1462-2920.15538
Schmalenberger A, Hodge S, Bryant A, Hawkesford MJ, Singh BK, Kertesz MA (2008) The role of Variovorax and other Comamonadaceae in sulfur transformations by microbial wheat rhizosphere communities exposed to different sulfur fertilization regimes. Environ Microbiol 10:1486–1500. https://doi.org/10.1111/j.1462-2920.2007.01564.x
Seo H, Kim S, Sagong HY, Son HF, Jin KS, Kim IK, Kim KJ (2016) Structural basis for cytokinin production by LOG from Corynebacterium glutamicum. Sci Rep 6:31390. https://doi.org/10.1038/srep31390
Shelomi M, Chen M-J (2020) Culturing-enriched metabarcoding analysis of the *Oryctes rhinoceros* Gut Microbiome. InSects 11:782. https://doi.org/10.3390/insects11110782
Shelomi M, Lin S-S, Liu L-Y (2019) Transcriptome and microbiome of coconut rhinoceros beetle (*Oryctes rhinoceros*) larvae. BMC Genomics 20:957. https://doi.org/10.1186/s12864-019-6352-3
Shobade SO, Zabotina OA, Nilsen-Hamilton M (2024) Plant root associated chitinases: structures and functions. Front Plant Sci. https://doi.org/10.3389/fpls.2024.1344142
Singh AV, Chandra R, Goel R (2013) Phosphate solubilization by Chryseobacterium sp. and their combined effect with N and P fertilizers on plant growth promotion. Arch Agron Soil Sci 59:641–651. https://doi.org/10.1080/03650340.2012.664767
Singh S, Tripathi DK, Singh S, Sharma S, Dubey NK, Chauhan DK, Vaculík M (2017) Toxicity of aluminium on various levels of plant cells and organism: a review. Environ Exp Bot 137:177–193. https://doi.org/10.1016/j.envexpbot.2017.01.005
Somssich M (2019) A short history of *Arabidopsis thaliana* (L.) Heynh. Columbia-0. PeerJ Preprints 7:e26931b26935. https://doi.org/10.7287/peerj.preprints.26931v5
Song S, Ee AWL, Tan JKN, Cheong JC, Chiam Z, Arora S, Lam WN, Tan HTW (2021) Upcycling food waste using black soldier fly larvae: effects of further composting on frass quality, fertilising effect and its global warming potential. J Clean Prod 288:125664. https://doi.org/10.1016/j.jclepro.2020.125664
Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a001438
Tai V, Carpenter KJ, Weber PK, Nalepa CA, Perlman SJ, Keeling PJ (2016) Genome evolution and nitrogen fixation in bacterial ectosymbionts of a protist inhabiting wood-feeding cockroaches. Appl Environ Microbiol 82:4682–4695. https://doi.org/10.1128/AEM.00611-16
Talwar C, Nagar S, Kumar R, Scaria J, Lal R, Negi RK (2020) Defining the environmental adaptations of genus Devosia: insights into its expansive short peptide transport system and positively selected genes. Sci Rep 10:1151. https://doi.org/10.1038/s41598-020-58163-8
Tan JKN, Lee JTE, Chiam Z, Song S, Arora S, Tong YW, Tan HTW (2021) Applications of food waste-derived black soldier fly larval frass as incorporated compost, side-dress fertilizer and frass-tea drench for soilless cultivation of leafy vegetables in biochar-based growing media. Waste Manag 130:155–166. https://doi.org/10.1016/j.wasman.2021.05.025
Tatusova T, DiCuccio M, Badretdin A et al (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569
Thongbunrod N, Chaiprasert P (2023) Anaerobic microbial cocktail of lignocellulolytic fungi and bacteria with methanogens for boosting methane production from unpretreated rice straw. Bioprocess Biosyst Eng 46:251–264. https://doi.org/10.1007/s00449-022-02829-2
Uriarte M (2000) Interactions between goldenrod (*Solidago altissima* L.) and its insect herbivore (*Trirhabda virgata*) over the course of succession. Oecologia 122:521–528. https://doi.org/10.1007/s004420050975
Vorhölter F, Wiggerich H, Scheidle H, Sidhu VK, Mrozek K, Küster H, Pühler A, Niehaus K (2012) Involvement of bacterial TonB-dependent signaling in the generation of an oligogalacturonide damage-associated molecular pattern from plant cell walls exposed to *Xanthomonas campestris* pv. campestris pectate lyases. BMC Microbiol 12:239
Wang X, Wu N, Wu X, Geng W, Xu X (2022) Effect of insect feces (*Hermetia illucens*) on rice growth and heavy metal migration from polluted soil to rice plant. Environ Sci Pollut Res Int 29:14695–14704. https://doi.org/10.1007/s11356-021-16803-0
Wantulla M, van Zadelhoff K, van Loon JJA, Dicke M (2022) The potential of soil amendment with insect exuviae and frass to control the cabbage root fly. J Appl Entomol 147:181–191. https://doi.org/10.1111/jen.13097
Watson C, Schlösser C, Vögerl J, Wichern F (2021) Excellent excrement? Frass impacts on a soil's microbial community, processes and metal bioavailability. Appl Soil Ecol 168:104110. https://doi.org/10.1016/j.apsoil.2021.104110
Wiegel J, Wilke D, Baumgarten J, Opitz R (1978) Transfer of the nitrogen-fixing hydrogen bacterium Corynebacterium autotrophicum Baumgarten et al. to Xanthobacter gen. nov. Int J Syst Bacteriol 28:573–581
Xu R, Zhang K, Liu P, Han H, Zhao S, Kakade A, Khan A, Du D, Li X (2018) Lignin depolymerization and utilization by bacteria. Bioresour Technol 269:557–566. https://doi.org/10.1016/j.biortech.2018.08.118
Yeager CM, Gallegos-Graves V, Dunbar J, Hesse CN, Daligault H, Kuske CR (2017) Polysaccharide degradation capability of actinomycetales soil isolates from a semiarid grassland of the colorado plateau. Appl Environ Microbiol. https://doi.org/10.1128/AEM.03020-16
Yu F, Gu T, Wei J et al (2023) CaFe-layered double hydroxide corn straw biochar reduced heavy metal uptake by *Brassica campestris* L. and *Ipomoea aquatic* F: rhizosphere effects and oxidative stress alleviation. J Environ Manag 330:117227. https://doi.org/10.1016/j.jenvman.2023.117227
Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y (2018) dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res 46:W95–W101. https://doi.org/10.1093/nar/gky418
Zhang Q, Zhang Y, Wang Y et al (2023) Effects of pruning on tea tree growth, tea quality, and rhizosphere soil microbial community. Microbiol Spectr. https://doi.org/10.1128/spectrum.01601-23

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.