Investigating the Use of Water Hyacinth (*Eichhornia crassipes*) to Enhance Soil Health in Agriculture

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Authored by: Adam Canning

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Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER) James Cook University
Townsville Phone: (07) 4781 4262 TropWATER@jcu.edu.au Web: www.jcu.edu.au/tropwater/

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Contacts

For more information contact: Dr Adam Canning (adam.canning@jcu.edu.au)

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1. EXECUTIVE SUMMARY

1. This study investigated the use of water hyacinth (*Eichhornia crassipes*) as an organic mulch for agricultural soils. Water hyacinth is a nutrient-rich, fast-growing invasive species, and its use as mulch could provide an alternative to traditional amendments. The research assessed its effects on soil microbial diversity, functional traits, and ecosystem services, such as nutrient cycling, organic matter decomposition, and pathogen suppression.
2. The study was conducted at three sites in Rockhampton, Queensland: a macadamia orchard, a lychee orchard, and a grazing pasture. Treatments included water hyacinth mulch, woody mulch, and no-mulch control, with and without urea application. Soil samples were analysed for microbial diversity, community composition, and functional traits.
3. Vegetation type strongly influenced microbial diversity and functional traits. Macadamia orchards supported higher microbial diversity and traits related to nutrient cycling and decomposition. Lychee orchards had reduced ectomycorrhizal diversity and shifts in decomposition traits, possibly due to competitive interactions or management practices.
4. Water hyacinth mulch increased microbial traits related to rapid nutrient cycling and decomposition, such as arbuscular mycorrhizal and chitinolytic diversity. Woody mulch favoured traits associated with organic matter stabilisation and nematode suppression. Woody mulch also increased the diversity of opportunistic human pathogens, raising biosecurity concerns.
5. Urea application increased arbuscular mycorrhizal diversity and enhanced decomposition traits. However, it also reduced the diversity of some microbial groups, such as methanotrophs and nematophages, under specific conditions. These results indicate trade-offs in nitrogen use.
6. Water hyacinth mulch improved soil microbial diversity and functions but showed variability depending on vegetation type and soil conditions. The results suggest that mulch type should be chosen based on specific agricultural and environmental needs.
7. The study was conducted over a short period and included a single application of mulch and nitrogen treatments, limiting observations of long-term effects. While metabarcoding can indicate functional differences, they do not guarantee that functional outcomes occurred, and further work would be required to measure potential functions and ecosystem services directly. The findings may also not be directly applicable to other soil types, climates, or agricultural systems.
8. Long-term experiments are required to evaluate the persistence of treatment effects and their cumulative impacts. Future studies should incorporate assessments of functional outcomes (e.g., decomposition rates or greenhouse gas emissions) to measure microbial activity and extend to different soil types, climates, and management practices, such as cover cropping and irrigation. This would help develop more generalised recommendations for soil health improvement.

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3. INTRODUCTION

Organic mulches and composts can improve soil health by helping to regulate soil temperature, reduce water evaporation, and minimize erosion by providing a protective cover over the soil surface. Composts, rich in decomposed organic matter, contribute to soil structure by increasing aggregate stability and porosity, promoting better aeration and water infiltration. As organic amendments are decomposed, they also supply nutrients such as nitrogen, phosphorus, and potassium in plant-available forms, boosting fertility while reducing reliance on synthetic fertilizers. Furthermore, organic amendments enhance microbial activity and biodiversity, which can help support processes like nutrient cycling, organic matter decomposition, and disease suppression. Organic inputs can also increase soil organic carbon, which not only improves soil structure but also contributes to long-term carbon sequestration.

While mulches from tree biomass or crop residues are common and generally readily available, they usually have relatively high carbon:nitrogen levels compared to non-lignin amendments or composts supplemented with nitrogen rich feedstocks. As an alternative, water hyacinth (*Eichhornia crassipes*), an invasive aquatic macrophyte, offers unique opportunities and challenges. With its rapid growth, high biomass production, and nutrient-rich composition, water hyacinth has the potential to be repurposed as a soil amendment.

Water hyacinth is widely recognized for its ecological and economic challenges. As one of the world's most invasive aquatic weeds, it clogs waterways, disrupts aquatic ecosystems, and hinders activities such as fishing and transportation. Attempts to manage its spread have often focused on herbicide spraying or harvesting and disposal, but these approaches have limited sustainability. An alternative, circular approach involves transforming harvested water hyacinth into a resource for agricultural systems. For example, composting, biochar production, and direct application as mulch have shown promise for repurposing this plant while addressing issues of waste and nutrient cycling. Despite these possibilities, questions remain about the effectiveness of water hyacinth-based amendments under different soil types, cropping systems, and environmental conditions.

Understanding how water hyacinth-based amendments influence soil processes is crucial if its use in agriculture is to be beneficial and sustainable. Soil microbial communities play a central role in mediating nutrient cycling, organic matter decomposition, and plant health. The diversity and composition of microbial communities, along with their functional traits, are indicators of soil ecosystem health and resilience. Investigating how these communities respond to water hyacinth amendments can provide insights into the mechanisms driving soil improvement and identify potential trade-offs. Furthermore, if the mulch alters soil nutrient cycling and this improves nutrient retention, then this may result in enhanced nutrient use efficiency and reduced environmental losses.

To address these gaps, this report presents a literature review and an experimental study focused on water hyacinth as a soil amendment. The literature review synthesizes existing evidence to explore where and how water hyacinth has been used as an agricultural amendment. Building on this, the experimental study examined the impacts of water hyacinth mulch and nitrogen application on soil health across three agricultural systems near Rockhampton, Queensland.

4. LITERATURE REVIEW

4.1 AIMS AND METHODS

This literature review that seeks to inform the following questions:

1. Where and how has water hyacinth been used a soil amendment?
2. How have water hyacinth-based soil amendments ameliorated soils via the alteration of soil structure, nutrient availability, and soil microbe assemblages?

To identify potentially relevant scientific publications, the following search string (examining all fields) was used to identify all records in the Web of Science database. This yielded 63 results, which were then further refined using their abstracts to determine their relevance to informing the above questions. Contaminant remediation uses were excluded.

Search string:

("water hyacinth" OR "Eichhornia crassipes") AND "Soil" AND ("amendment" OR "conditioner" OR "improvement" OR "structure" OR "texture" OR "porosity" OR "nutrients" OR "microb*") AND "agricultur*"

4.1 FINDINGS

4.1.1 MULCH AND NON-COMPOST FERTILISER

The use of water hyacinth (*Eichhornia crassipes*) and other organic materials as mulch or non-compost fertilisers has been investigated in various cropping systems. These studies provide insights into their effects on soil fertility, nematode suppression, and plant growth.

Water hyacinth appears to have potential as an amendment to improve soil fertility and crop growth. Hernández-Fernández et al. (2024) evaluated different quantities of dried water hyacinth in combination with red ferralytic soil for cultivating common chilli (*Capsicum annuum*) in Cuba. The addition of 400 g of dried water hyacinth to the substrate resulted in significantly greater fruit yield (7.5 ± 1.8 fruits/plant) compared to the non-amended control (0.2 ± 0.2 fruits/plant). Higher application rates did not yield any additional benefits. This suggests that water hyacinth may enhance nutrient availability or improve soil conditions. However, the study lacked detailed information on the nutritional value of the water hyacinth or the physicochemical properties of the soil, limiting insight into the underlying mechanisms for the improved plant performance.

Majee et al. (2019)also reported increased plant growth when using an organic fertiliser composed of water hyacinth, rice husk ash, and steamed bone meal for growing potted marigold (*Tagetes* spp.) in India. The amended soils produced plants with greater length compared to the control. However, the absence of treatment replication and elemental analysis considerably limits the reliability of the findings and their applicability.

In addition to improving crop growth, water hyacinth has also been trialled as a mulch for pest suppression. Khan et al. (2022) examined the use of mulch made from billygoat weed (*Ageratum conyzoides*) or water hyacinth (*Eichhornia crassipes*) and the inoculation of the nematophagous fungus *Pochonia chlamydosporia*, to control root-knot nematode (Meloidogyne incognita) in chickpea (*Cicer arietinum*). The study, conducted under glasshouse conditions in India, found that the combined application of *P. chlamydosporia* and water hyacinth mulch resulted in a significant reduction in the number of galls (14.20 ± 1.41) compared to the control (112 ± 7), although it was less effective than billygoat weed (*Ageratum conyzoides*) mulch. Despite water hyacinth being the

least effective treatment, all amended treatments outperformed the control, demonstrating that water hyacinth mulch can contribute to nematode management.

Ramdas et al. (2017) investigated the medium-term effects of various organic and inorganic nutrient sources on soil organic carbon (SOC), carbon accumulation, and microbial and enzyme activities in flooded rice plots in India. Treatments included vermicompost, glyricidia and eupatorium (GE), dhaincha (SR), farmyard manure (FYM), a mix of dry paddy straw and water hyacinth (PsWh), and mineral fertilisers. The PsWh treatment notably increased SOC levels, comparable to those achieved with FYM and exceeding untreated controls as well as GE and SR treatments. The PsWh treatment also showed an increase in soil microbial biomass, greater than GE, SR, and mineral fertilisers, though FYM remained superior in supporting microbial biomass. In terms of microbial efficiency, PsWh exhibited a lower metabolic quotient compared to mineral fertilisers and untreated controls, suggesting a more efficient microbial community under PsWh. Enzyme activities, particularly dehydrogenase and phosphatase, were elevated in the PsWh treatment. While dehydrogenase activity was lower than FYM, it surpassed levels found in mineral fertilisers, GE, and untreated controls. Phosphatase activity was high, second only to FYM, and higher than that observed in soils treated with vermicompost, GE, SR, and the control. Urease activity was similar across most treatments, with PsWh showing comparable levels to FYM. Combining paddy straw and water hyacinth enhances SOC, microbial biomass, and enzyme activities, often outperforming other amendments. The increased phosphatase activity may improve phosphorus mineralisation, addressing phosphorus's tendency to bind with soil particles and become less available to plants. Typically, only a small fraction of applied phosphorus remains plant-available, depending on soil characteristics. Phosphatase facilitates the release of bound phosphorus, improving availability for crops. If water hyacinth reliably elevates phosphatase, it may be a useful tool in nutrient management, particularly for improving phosphorus availability.

4.1.2 BIOCHAR AND OTHER ENGINEERED PRODUCTS

Recent studies on water hyacinth (WH) biochar have demonstrated its potential for enhancing soil fertility, moisture retention, and microbial health, with findings indicating that specific properties of biochar—such as cation exchange capacity (CEC), carbon stability, water-holding capacity, and nutrient content—are significantly influenced by pyrolysis temperature and biochar composition (Table 1 & 2).

Studies by Gezahegn et al. (2024) and Bao et al. (2021) found that low-temperature pyrolysis (300–400°C) produced WH biochar with higher CEC and nitrogen content than biochar made from other materials, such as wood or agricultural residues. This high CEC makes WH biochar effective in nutrient-poor soils by enhancing nutrient retention. For example, WH biochar's C/N ratio at 350°C was markedly lower than those of biochars from woody feedstocks, supporting faster nutrient cycling and more immediate fertility benefits. However, as pyrolysis temperature increased to 550–750°C, WH biochar demonstrated greater stability, with low H/C ratios indicative of high carbon stability due to fused aromatic ring structures. At 750°C, WH biochar had the highest water-holding capacity and increased pore volume, outperforming most woody or fibrous biochars, which tend to have smaller pore volumes and lower water retention. Gezahegn et al (2024), however, noted that these high-temperature biochars, while more stable, might have a slower nutrient release, suggesting a trade-off between stability and immediate fertility enhancement.

Khatun et al. (2024) compared WH biochar with biochars derived from rice straw, sawdust, and a mixed feedstock blend (1:1:1) produced at 400°C. WH biochar had superior water-holding capacity,

surface area, and nutrient content, including phosphorus, potassium, sulfur, calcium, and zinc, compared to biochar from the other feedstocks. WH biochar's water-holding capacity outperformed rice straw and sawdust biochars, which are typically low in available nutrients and water retention. These findings suggest that WH biochar offers a dual benefit of nutrient and moisture enhancement, potentially reducing the need for separate soil amendments.

The liming potential of water hyacinth (WH) biochar was highlighted in Jutakanoke et al. (2023), who investigated its application in acidic sulfate soils in Rangsit, Thailand. WH biochar, with a pH of 7.62, improved water spinach (*Ipomoea aquatica*) growth in these conditions, yielding greater plant height and biomass compared to unamended soils. The study also examined changes in soil microbial communities using 16S amplicon sequencing. WH biochar-amended soils had higher populations of *Bacillus*, *Paenibacillus*, and *Sphingomonas*, beneficial bacteria known for promoting plant growth through mechanisms like phytohormone production, phosphorus solubilization, and nitrogen fixation. In unamended soils, *Ktedonobacterales* were three times more prevalent, while *Bacillus* was twice as abundant in biochar-treated soils. Without comparisons to other biochars or soil amendments, the study offers limited insight into WH biochar's performance against other liming amendments.

In humid, high-rainfall conditions He et al. (2022), tested compost-biochar mixtures with varying WH biochar content (15%, 30%, and 45%) and compared these to compost alone. WH biochar treatments helped stabilise soil pH and increased soil electrical conductivity, indicating improved nutrient exchange. The 45% WH biochar treatment maintained pH levels with minimal fluctuation, in contrast to compost-only treatments, where pH dropped substantially. The increased conductivity in WH biochar-treated soils suggested greater nutrient availability, although there was no assessment of potential salt accumulation from the elevated conductivity, a potential risk for plant growth in the long term.

The impact of WH biochar on water retention and soil structure has also been examined. Bao et al. (2021) showed that WH biochar produced at 300°C retained 79.07% water, outperforming biochar produced at 600°C (41.29%) and biochars from wood and chicken manure, which typically held 10–20% water. Huang et al. (2021) tested WH biochar in sandy soils at two temperatures (300°C and 600°C) and found that 10% WH biochar at 300°C increased water retention by 371% compared to non-amended soil, which exceeded the 5–20% retention gains from wood and chicken manure biochars. Mei et al. (2021) investigated WH biochar's influence on soil cracking and moisture retention through drying-wetting cycles, finding WH biochar reduced soil cracking and improved water retention at all temperatures, with the highest retention at 700°C. In addition, Garg et al. (2020) assessed WH biochar in sandy soils at varying compaction levels and observed that 10% WH biochar provided optimal water retention under low compaction, with benefits decreasing at higher compaction levels. Bordoloi et al. (2018) also found that a 15% WH biochar application improved water retention to around 48%, compared to 29.5% in unamended soils, while reducing soil cracking across drying-wetting cycles. Collectively, WH biochar demonstrates superior water retention and structural stability across a range of conditions and is especially effective at low pyrolysis temperatures, high application rates, and in sandy, low-compaction soils. However, all studies lacked field-based validation, leaving questions about long-term effectiveness.

The polymer hydrogel created by Rop et al. (2019) using water hyacinth fibres presented a different approach, where the material, rather than acting as a soil amendment, was designed to enhance soil moisture-holding capacity. The hydrogel enhanced the soil's moisture-holding capacity significantly, with moisture retention increasing from 35% in unamended soil to 68% when amended with 1.5% copolymer by weight. This suggests potential benefits in water-limited regions where soil moisture

retention is critical. However, as a synthetic material, this cellulose-graft-poly (ammonium acrylate-co-acrylic acid) hydrogel might introduce microplastic contaminants if it degrades over time, a concern not associated with natural biochar amendments. While the authors did not examine the potential environmental impact of microplastic residues, such considerations are increasingly relevant given the known persistence and ecological risks of synthetic polymers. Further studies are warranted to assess the long-term decomposition of this hydrogel in soil environments and its suitability for sustainable agricultural applications.

Table 1. A comparison of properties between water hyacinth biochar produced at low or high temperatures.

Property	WH Biochar (300-400°C)	WH Biochar (550-750°C)	Other Biochars (Wood, Rice Straw, Chicken Manure)
C/N Ratio	Low	Very Low	Typically higher, slower nutrient cycling
Nitrogen Content (%)	High	Low	Lower than WH biochar, except chicken manure biochar
Cation Exchange Capacity	High	Moderate	Moderate, less effective for nutrient retention
pH	Neutral to Alkaline	Neutral	Varies, often lower than WH biochar for liming
Water-Holding Capacity	Very High	High	Moderate, typically higher in WH biochar
Surface Area (m^2/g)	Moderate	High	Varies, usually lower than high-temperature WH biochar
Phosphorus (P)	Moderate to High	Low to Moderate	Generally lower than WH biochar
Potassium (K)	Low to Moderate	Moderate	Moderate, similar to WH biochar
Calcium (Ca)	Moderate	High	Often lower than WH biochar, especially at higher temperatures
Electrical Conductivity	Moderate	Moderate to High	Varies, generally lower than WH biochar

Table 2. Summary of nutrient compositions for the different water hyacinth (WH) feedstocks and biochars reported in studies in this section. ND is not detected.

Element/Property	Dried Biomass (Khatun et al. 2024)	WH Biochar 300°C (Bao et al. 2021)	WH Biochar 600°C (Bao et al. 2021)	WH Biochar (He et al. 2022)	WH Biochar (Khatun et al. 2024)
Volatile Matter (VM %)	74.70 ± 1.04	-	-	-	20.05 ± 0.54
Fixed Carbon (%)	8.03 ± 0.58	-	-	42.48 ± 0.41	-
Carbon (C, %)	-	42.78	66.35	43.37	-
Nitrogen (N, %)	0.78 ± 0.14	-	-	2.72	1.38 ± 0.20
Phosphorus (P, g Kg^{-1})	5.48 ± 0.28	2.65	1.07	-	10.67 ± 0.51
Potassium (K, g Kg^{-1})	1.46 ± 0.26	3.82	6.22	-	5.21 ± 0.15
Sulfur (S, %)	4.63 ± 0.02	0.09	0.14	0.16	6.62 ± 0.29
Sodium (Na, g Kg^{-1})	1.1 ± 0.2	3.43	0.39	-	1.97 ± 0.02
Calcium (Ca, g Kg^{-1})	10.33 ± 1.16	6.28	8.29	-	31.67 ± 1.44
Magnesium (Mg, g Kg^{-1})	6.4 ± 0.92	3.97	0.67	-	25.50 ± 2.60
Zinc (Zn, g Kg^{-1})	42.01 ± 0.80	-	-	9.11	184.45 ± 0.93
Oxygen (O, %)	-	22.08	10.16	37.82	-
Chlorine (Cl, %)	-	7.87	6.09	-	-
Aluminium (Al, %)	-	0.04	0	-	-
Silicon (Si, %)	-	0.15	0.14	-	-
Rhodium (Rh, %)	-	0.33	0.13	-	-
Boron (B, %)	-	3.44	0	-	-
Tellurium (Te, %)	-	0.31	0	-	-
Manganese (Mn, %)	-	0	0.37	-	-
Total (%)	-	97.24	100.02	-	-
Water Holding Capacity (WHC, mL g^{-1})	-	-	-	-	4.77 ± 0.05
Yield (%)	-	-	-	-	37.80 ± 0.50
Surface Area (m^2 g^{-1})	-	-	-	-	205.40 ± 3.2
pH	-	-	-	-	8.06 ± 0.02
Electrical Conductivity (EC, dS m^{-1})	-	-	-	-	13.03 ± 0.32
Cation Exchange Capacity (CEC)	-	-	-	-	35.56 ± 0.00
Arsenic (As, mg Kg^{-1})	-	-	-	2.29	-
Cadmium (Cd, mg Kg^{-1})	-	-	-	ND	-
Chromium (Cr, mg Kg^{-1})	-	-	-	10.77	-
Copper (Cu, mg Kg^{-1})	-	-	-	2.41	-
Lead (Pb, mg Kg^{-1})	-	-	-	1.48	-
Mercury (Hg, mg Kg^{-1})	-	-	-	ND	-
Nickel (Ni, mg Kg^{-1})	-	-	-	22.45	-
Selenium (Se, mg Kg^{-1})	-	-	-	ND	-

4.1.3 COMPOST

Patra et al. (2022) composted five types of organic was viz., leaf litter (*Tectona grandis*), water hyacinth (*Eichhornia crassipes*), cauliflower waste (*Brassica oleracea* var. botrytis), coir pith, and mushroom spent with and without the use of earthworm (*Eisenia fetida*). All processed raw materials were thoroughly mixed with cow dung in a proportion of 5:1 prior to composting, with four replicates of each treatment. Final matured compost and vermicompost were harvested at 150 days of decomposition. In comparing the water hyacinth based compost and vermicompost, nutrient compositions were similar; while the vermicompost having greater bacteria abundance and lower fungi abundance than compost. The bacteria-to-fungi ratio in soil is a critical indicator of soil health, influencing fertility, structure, and ecosystem productivity. Bacteria rapidly decompose simple organic compounds, facilitating quick nutrient cycling, while fungi break down complex materials, aiding in long-term nutrient stability and enhancing soil structure through their mycelial networks. This ratio also affects carbon cycling, with fungal dominance promoting more stable carbon sequestration. In general, soil disturbances such as tilling promote bacterial dominated soil assemblages, while stable and lignin-rich soils promote greater fungal abundance.

Shyam et al (2022) examined whether water hyacinth compost can be improved by the addition of pond sediment as a bulking agent. They compared three treatments: (1) water hyacinth without pond sediment; (2) 1:5 mixture of pond sediment to water hyacinth; and (3) a 1:2 mixture of pond sediment to water hyacinth. Prior to composting, the water hyacinth was rinsed, sun dried for 24h, and mulched into 3-5 cm fragments. They found the 1:5 mixture yielded a compost with greatest level of available nutrients. They also cautioned that composts using aquatic macrophytes and pond sediments should be tested for the presence of heavy metals prior to field application. Nutrients within water hyacinth and pond sediment are likely to vary between locations, potentially limiting the wider applicability of this study. The nutrient contents of the raw materials and composts are in Table 3.

Bhatti et al (2021) experimentally examined the effect of different compost mixtures on the growth and macronutrient concentrations in fodder maize (*Zea mays* L. cv. Akbar) in Tandojam, Pakistan. The experimental treatments, each replicated four times, consisted of: Control (No amendment), Recommended NPK, Water Hyacinth Compost, Fruits + Vegetables Compost, and Banana Leaves Compost, with each compost type applied at a rate of 15 tons per hectare via ploughing into 4 m x 4 m plots. The soil used in the experiment was fine-textured (clayey), slightly alkaline, non-saline, low in organic matter, calcareous, deficient in nitrogen, marginal in phosphorus, and had sufficient potassium levels. Composts were made by blending organic products with cattle manure at a 3:1 ratio, and turned every 15 days for three months, followed by one month of stablisation and drying. Seeds were sown using a hand drill and experiment terminated 90 days after sowing. No information was provided on the source of the water hyacinth or the final elemental composition of the compost produced. Adding composts and NPK fertilizer significantly boosted growth and yield indicators in maize, with plant height increasing by up to 26%, the number of leaves per plant by up to 20%, stem girth by up to 22%, and fresh weight of maize fodder by up to 25%. These treatments also elevated the levels of key macronutrients in maize leaves, with nitrogen increasing by up to 46%, phosphorus by up to 27%, and potassium by up to 38% compared to the control. There was no notable difference among the various compost treatments and NPK fertilizer in terms of these growth and yield parameters, except for the phosphorus concentration in maize leaves. Additionally, significant increases in macronutrient concentrations were observed in both surface and subsurface soil where inorganic and organic amendments were applied, compared to control plots. Given that composting water hyacinth typically requires an additional carbon feedstock to reach an optimal C/N ratio, and

that the C/N ratio cattle manure can vary considerably with it often being low, it is unknown whether an additional carbon feedstock was used in the composting of water hyacinth. The lack of detail describing the compost used make it very difficult to ascertain the extent to which the process used to compost water hyacinth affected the findings and how readily transferable these findings are.

Goswami et al. (2017) experimentally compared the use of drum composted water hyacinth, livestock manure, and vermicompost, as amendments to a clay-loam growing intensively cultivated tomato and cabbage crops for 80 days with an NPK (N-P-K=75-60-60 kg ha^{-1}) fertiliser in Tezpur, India. Changes in soil quality were indicated by assessing total and available nitrogen, total organic carbon, Bray's phosphorus, exchangeable potassium, microbial biomass, the degree of humification (aromatic C/aliphatic C), and the bioavailability of metals (Mg, Fe, Zn, Mn, Cu, Pb, Cd, and Cr). While crop quality was indicated by assessing leaf count, plant height, leaf area index, yield, nutrient density, and metal concentrations. The water hyacinth drum compost was made using a wet weight mixture of six parts water hyacinth, three parts cattle manure and one part sawdust, mixed in a rotary bin and composted for 30 days. Table 3 compares the nutrient and heavy metal concentrations in the resulting water hyacinth drum compost, vermicompost, manure, and soil. While soils amended with WH compost, vermicompost, and manure, had similar nutrient concentrations, the yield, shelf life and pericarp thickness were significantly greater in tomatoes grown with WH compost and vermicompost relative to those amended with manure. While the size and shape of cabbages were significantly greater in soils with the WH compost and vermicompost relative to those amended with manure. Comparatively, the water hyacinth drum compost had the highest concentrations of heavy metals, likely due to uptake from the waterway during plant growth, and this lead to greater bioavailability of metals in WH compost treated soils and the crops grown.

Yadav & Garg (2013) experimentally examined the performance of earthworms in vermicompost produced using eight different bedding material mixture combinations composed of water hyacinth, parthenium, and cow dung. Mixtures containing water hyacinth (WH) were Vermibins 2, 3, 4, and 8, while those without WH were Vermibins 1, 5, 6, and 7. Vermibin 2 (25% CD, 25% FIS, 50% WH) achieved a maximum biomass of 830 mg $worm^{-1}$, with a net biomass gain of 680 mg $worm^{-1}$ and a growth rate of 10.70 mg $worm^{-1}$ d^{-1}. Vermibin 3 (50% CD, 25% FIS, 25% WH) reached a maximum biomass of 810 mg $worm^{-1}$, a net gain of 630 mg $worm^{-1}$, and a growth rate of 11.25 mg $worm^{-1}$ d^{-1}. Vermibin 4 (25% CD, 50% FIS, 25% WH) showed similar performance, with a maximum biomass of 781 mg $worm^{-1}$ and a net gain of 601 mg $worm^{-1}$. Vermibin 8 (25% CD, 25% FIS, 25% WH, 25% PH) performed the best, with a maximum biomass of 980 mg $worm^{-1}$, a net gain of 715 mg $worm^{-1}$, and a growth rate of 12.76 mg $worm^{-1}$ d^{-1}. In contrast, Vermibins without WH showed varying performance. Vermibin 1 (100% CD) achieved the highest maximum biomass among non-WH bins with 990 mg $worm^{-1}$, a net gain of 823 mg $worm^{-1}$, and a growth rate of 16.97 mg $worm^{-1}$ d^{-1}. Vermibin 5 (25% CD, 25% FIS, 50% PH) reached a maximum biomass of 801 mg $worm^{-1}$, a net gain of 626 mg $worm^{-1}$, and a growth rate of 9.93 mg $worm^{-1}$ d^{-1}. Vermibin 6 (50% CD, 25% FIS, 25% PH) showed a maximum biomass of 842 mg $worm^{-1}$, a net gain of 680 mg $worm^{-1}$, and a growth rate of 10.70 mg $worm^{-1}$ d^{-1}. Vermibin 7 (25% CD, 50% FIS, 25% PH) had the lowest performance, achieving a maximum biomass of 739 mg $worm^{-1}$ and a net gain of 555 mg $worm^{-1}$, with a growth rate of 8.80 mg $worm^{-1}$ d^{-1}. In terms of reproductive performance, Vermibin 8 with WH had the highest cocoon production (356 cocoons), with 8.9 cocoons produced per worm, leading to 121 hatchlings and a total hatchling biomass of 25.2 g. Vermibin 2 also performed well, producing 284 cocoons, while Vermibin 3 produced 360 cocoons. Vermibin 4 had a moderate cocoon production (196 cocoons). Among the non-WH bins, Vermibin 1 produced the most cocoons (388), followed by Vermibin 6 (312 cocoons), while Vermibin 7 produced the fewest cocoons (152). Overall, mixtures with water

hyacinth demonstrated enhanced biomass production, growth rates, and reproductive success compared to those without WH.

Table 3. Summary of nutrient compositions for the different feedstocks and composts reported in studies in this section.

Factor	Patra et al. (Initial)	Patra et al. (Compost)	Patra et al. (Vermicompost)	Shyam et al. (Water Hyacinth)	Shyam et al. (ET1)	Shyam et al. (ET2)	Shyam et al. (ET3)	Goswami et al. (Drum Compost)	Goswami et al. (Vermicompost)	Goswami et al. (FYM)	Yadav & Garg (Water Hyacinth)
pH	-	-	-	-	-	-	-	6.9	6.4	6	7.1
EC (dS ma^{-1})	-	-	-	-	-	-	-	-	-	-	2.2
TOC (%)	-	-	-	2.6	6.88	2.71	1.74	3.8	4.6	2.7	31.27
TKN (%)	1.75	1.91	1.99	1.23	0.5	1.01	0.67	0.38	0.76	0.5	6.8
Total P (%)	0.29	0.32	0.37	0.1	0.14	0.35	0.17	0.15	0.25	0.15	5.75
Total K (%)	1.61	1.77	1.83	2.69	0.06	0.26	0.13	0.2	0.3	0.2	5.1
C:N Ratio	31	16	14	2.11	14.6	4.72	3.07	-	-	-	45.9
C:P Ratio	190	97	76	-	-	-	-	-	-	-	80.82
OM (%)	-	-	-	-	-	-	-	-	-	-	53.7
Fe (mg/kg)	7203	10080	10385	-	-	-	-	363	14	17	448
Cu (mg/kg)	14	30	35	-	-	-	-	78.1	5.6	2.1	221.9
Cd (mg/kg)	-	-	-	-	-	-	-	1.12	-	-	0.06
Zn (mg/kg)	62	105	113	-	-	-	-	73.4	12.2	10.1	315
Pb (mg/kg)	-	-	-	-	-	-	-	74.3	26.2	22.1	0.16
Mn (mg/kg)	1002	1526	1697	-	-	-	-	56.6	7.5	4.5	-
Av. N (mg/kg)	-	-	-	-	-	-	-	39	47	74	-
Av. P (mg/kg)	-	-	-	-	-	-	-	35	97	39.1	-
Av. K (mg/kg)	-	-	-	-	-	-	-	616	107	100	-
WHC (%)	-	-	-	-	-	-	-	58.4	63.21	59.11	-
HAC (%)	-	-	-	-	-	-	-	0.7	1.1	0.7	-
FAC (%)	-	-	-	-	-	-	-	1.2	2.3	1.4	-
COD	1040	687	633	-	-	-	-	-	-	-	-
CEC	76	105	118	-	-	-	-	-	-	-	-
S (%)	-	0.39	0.43	-	-	-	-	-	-	-	-
NH4+ (mg/kg)	350	290	270	-	-	-	-	-	-	-	-
NO3- (mg/kg)	80	1050	1100	-	-	-	-	-	-	-	-
NI	4.4	0.3	0.3	-	-	-	-	-	-	-	-
Urease	-	~1000	~1400	-	-	-	-	-	-	-	-
Dehydrogenase	-	~1000	~1300	-	-	-	-	-	-	-	-
Bacteria pop (x10^6)	-	6.6	20.5	-	-	-	-	-	-	-	-
Fungi pop (x10^6)	-	83	41	-	-	-	-	-	-	-	-
Actinomycetes pop (x10^6)	-	12.4	85.2	-	-	-	-	-	-	-	-
MBC (mg/g)	-	22	32.8	-	-	-	-	-	-	-	-

4.1.4 EXTRACTS AND ISOLATES

Elgala et al (2022) experimentally assessed the efficiency of aqueous water hyacinth shoot extract as a source of nutrients foliar sprayed to tomato plants. The efficacy was compared alongside plants grown without any foliar spray and plants sprayed with a commercial synthetic solution. All treatments also had a baseline soil application of superphosphate (144 kg ha^{-1}) and a fertigation three-part delivery of ammonium nitrate (total 360 kg ha^{-1}). The experiment was conducted during the summer of 2019 in a greenhouse in Qalubia Governorate, Egypt. In preparing the aqueous water hyacinth extract, the roots were removed due to heavy metal toxicity, and only shoots were used. The extract properties and nutrient concentrations are in Table 4. They found that water hyacinth extract treated planted had fresh and dry weights and fruit yields that were greater than the no-spray control, 37.5, 56.8 and 72.2%, respectively. The water hyacinth extract application increased the net return of tomato cultivation by approximately 1.84 times compared with the conventional practice (control).

Kato-Noguchi et al. (2014) examined the possible allelopathic effects of extracts and isolated allelopathic substances in water hyacinth on the growth of cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), timothy (*Phleum pratense*) and ryegrass (*Lolium multiflorum*). In all instances, the growth of roots and shoots were reduced, with increasing extract concentrations having more severe stunting. Using choromatography, the main allelopathically active substance was loliolide. As such, water hyacinth extract may be useful as a soil additive to control weeds.

Table 4. The chemical composition (concentrations in mg L^{-1}) of the extract produced by Elgala et al (2022)

Parameter	Value
pH	7.45
EC (dS m^{-1})	1.75
N	24.1
P	35.4
K	130
Fe	1.25
Mn	1.32
Zn	0.12
Cu	0.04
Pb	<1.50
Co	<0.20
Cd	<0.10
As	0.20
Se	0.19

5. FIELD EXPERIMENT

5.1 AIMS

This study aimed to investigate the effects of water hyacinth (WH) mulch, compared to a woody mulch and a no-mulch control, on soil microbial communities, functional traits, and nitrogen retention across three distinct agricultural settings: a macadamia farm, a lychee orchard, and a livestock grazing pasture near Rockhampton, Queensland. By including three distinct land use settings, the study seeks to determine if the effects of mulch and nitrogen treatments on microbial communities and nitrogen retention are consistent across different agricultural contexts or reveal system-specific interactions based on unique soil and management conditions. Specifically, the research aimed to:

1. Assess the impact of mulch type and nitrogen addition on soil microbial alpha and beta diversity, as well as on microbial community composition. This included examining how treatments influence overall diversity and community structure.
2. Examine the effects of mulch type and nitrogen addition on microbial functional trait alpha and beta diversity, focusing on traits related to key soil ecosystem services (nutrient cycling, organic matter decomposition, pollutant degradation, and plant health). This aim also explores interactions across the three land use settings to determine how these traits respond to treatments in different agricultural contexts.

5.2 METHODS

5.2.1 STUDY SITES AND EXPERIMENTAL DESIGN

The experiment was conducted across three agricultural sites near Rockhampton, Queensland: a macadamia farm, a lychee orchard, and a livestock grazing pasture. At each site, 2 × 2 m plots received either water hyacinth (WH) mulch, woody mulch, or a no-mulch control, arranged in a randomized layout. At the macadamia and lychee orchards, plots were positioned halfway between trees along orchard rows, while at the grazing site, livestock were excluded from the plots. WH mulch used raw water hyacinth that was sourced from Murray Lagoon near Rockhampton, pressed and mulch to remove excess water and reduce size, then solarised under a tarp for four weeks. Woody mulch was provided by Rockhampton Regional Council from their vegetative waste. Mulch was applied in a 5 cm layer, with half of the plots additionally receiving 50 kg N/ha of 15N-labeled urea (5%) dissolved in 2L of MilliQ water and evenly sprayed over each plot as a fine mist to ensure minimal spillover.

Each treatment combination was replicated five times with treatments randomly assigned. Mulch and urea treatments were applied on 30 May 2024, and left undisturbed for seven weeks, after which composite soil samples were taken on 18 July 2024. Soil samples consisted of five randomly collected soil cores per plot, taken to a depth of 20 cm, mixed, and immediately chilled for transport to the laboratory. Laboratory analysis included assessments of soil chemistry, 15N retention, and microbial community structure and functional traits through metabarcoding. This experimental design facilitated examination of how mulch type, nitrogen application, and land use influenced microbial community composition, functional traits, and nitrogen retention across varied management contexts.

5.2.1 SOIL PHYSICOCHEMISTRY

To assess the physicochemical properties of soils across the experimental treatments, samples were sent to Nutrient Advantage, a NATA-accredited and ASPAC-certified Australian laboratory

specializing in soil and plant tissue nutrient testing. This accreditation ensures compliance with the AS/ISO 17025 standard, confirming the laboratory's technical competence, accuracy, and traceability of results.

At Nutrient Advantage, a comprehensive suite of soil parameters was analysed. The tests conducted included measurements of pH (1:5 water and 1:5 $CaCl_2$), electrical conductivity (EC) and EC saturation index, and key nutrients such as nitrate nitrogen, ammonium nitrogen, and available phosphorus (Colwell method with phosphorus buffer index). Cations were analysed using ammonium acetate extraction for calcium, potassium, magnesium, and sodium, with the calcium-to-magnesium ratio also calculated. Additionally, available potassium was measured through ammonium acetate extraction. Trace elements, including copper, iron, manganese, and zinc, were assessed via DTPA extraction, along with boron and sulphur using KCl40 extraction. Chloride levels were also measured. The analysis further included total carbon and total nitrogen (both via combustion) to assess organic matter content, alongside the carbon-to-nitrogen (C:N) ratio. Other parameters measured included cation exchange capacity (CEC) including aluminium, sodium percentage of CEC, and aluminium percentage of CEC, which provide insights into soil salinity, structure, and nutrient-holding capacity. Soil colour and texture were also recorded to further characterize the soil matrix.

Redundancy Analysis (RDA) was performed to assess the influence of vegetation type (Veg), mulch treatment (Mulch), and urea application (Urea), including the interaction between mulch and urea, on soil physicochemical properties. The anova.cca function was used to perform a permutation-based ANOVA test (999 permutations) to evaluate the significance of each term.

To identify significant soil parameters, the envfit function from the vegan package was applied (Dixon and Palmer 2003; Oksanen et al. 2007), producing environmental vectors filtered for high correlation strength ($r > 0.8$) and statistical significance ($p < 0.05$). Significant vectors were further simplified using hierarchical clustering based on Euclidean distance. Clusters were defined by cutting the dendrogram into two groups to condense related parameters, which reduced redundancy in the final RDA visualization. Analysis conducted in R 4.4.1 (R Core Team 2024).

5.2.2 DNA EXTRACTION, PROCESSING & METABARCODING

Soil samples from each plot were sent to Metagen Australia for metabarcoding of bacteria, fungi and nematodes (using 16S, 18S, and nematode-specific genes respectively), using the following methods in this section:

DNA was extracted from 10 g soil subsamples using a modified version of the modular universal DNA extraction protocol (Sellers et al. 2018). Soil was homogenized with garnet sand and lysis buffer using a SPEX 2010 Geno Grinder (SPEX SamplePrep, NJ) at 1700 strokes per minute for 5 minutes. After centrifugation, the supernatant was treated with a flocculant to remove humic acid contaminants, centrifuged again, and DNA was recovered from 10 ml of the supernatant using SPRI beads (Oberacker et al. 2019). DNA quality and concentration were assessed using the Quantifluor dsDNA system (Promega, MI) and gel electrophoresis to ensure adequate yield and purity for subsequent metabarcoding.

Eukaryotic and bacterial/archaeal communities were characterized through metabarcoding. The primer sets NF1/18S2rB (Porazinska et al. 2009) and Pro341F/Pro805R (Takahashi et al. 2014) were used to amplify 18S and 16S rRNA genes, respectively, for eukaryotes and prokaryotes, while Nemf/18Sr2b (Sikder et al. 2020) targeted soil nematodes. DNA amplification was conducted in two

stages, following the Illumina protocol for dual-indexed amplicons to enable sample multiplexing. In the first PCR, 25 cycles amplified target regions; in the second, 15 cycles incorporated dual indexes for each sample. Fluorimetry (Quantifluor dsDNA) was used to standardize the concentration of final amplicons, which were then pooled at equimolar concentrations, purified with SPRI beads, normalized to 10 nM, and sequenced on an Illumina MiSeq (2 x 300 bp) at the IMB Sequencing Facility, University of Queensland.

Raw sequences were demultiplexed with DeML (Renaud et al. 2015). Amplicon sequence variants (ASVs) were generated using DADA2 (Callahan et al. 2016) in R version 3.5.1. For 18S and 16S reads, forward and reverse reads were truncated at 270 bp and 240 bp, respectively, with stringent error thresholds (2 expected errors for forward, 3 for reverse in 16S; 3 for forward, 4 for reverse in 18S) to minimize erroneous sequences. Chimeras were identified and removed using DADA2's "removeBimeraDenovo" function with the "consensus" method. Taxonomy was assigned to genus level for 16S using the Silva database version 128 (Quast et al. 2013) and to species level for 18S using the PR2 database version 4.12 (Guillou et al. 2013).

5.2.3 DATA ANALYSIS

Using the microeco package in R 4.4.0 (Liu et al. 2021; R Core Team 2024), all raw ASV read data was analysed to examine how the genera and functional trait alpha and beta diversity and compositions of bacteria, fungi, and nematode, assemblages differ between mulch, urea, and vegetation treatments. Prior to analysis, datasets were transformed using the regularized logarithm (rlog) transformation in the DESeq2 package (Love et al. 2014). This transformation stabilizes variance across samples with different sequencing depths, preserves low-abundance taxa, and maintains the relative abundance structure, unlike rarefying, which discards a portion of the data and may introduce noise. As a result, the rlog transformation provides a more robust representation for statistical comparisons.

Genera alpha diversity was indicated by the Shannon's Diversity index, calculated using the vegan package dependency (Dixon and Palmer 2003; Oksanen et al. 2007). Analysis of variance (ANOVA, type II) was used to test the effects of mulch type and urea application (and its interaction), and land use type on alpha diversity, using the car package (Fox et al. 2012). Genera beta diversity was assessed using Bray-Curtis dissimilarity and distance-based redundancy analysis (db-RDA), also in the vegan package (Dixon and Palmer 2003; Oksanen et al. 2007). The anova.cca function was used to perform a permutation-based ANOVA test to determine the effects of mulch type, urea application, their interaction, and land use type on beta diversity, following the approach recommended by Legendre et al. (2011). Differential abundance analysis of microbial taxa was conducted using DESeq2 (Love et al. 2014). DESeq2 was chosen for differential abundance analysis of microbial taxa due to its robust handling of count-based data, its ability to model and normalize data with varying sequencing depths, and its effectiveness in identifying significant differences while controlling for false discovery rates, making it particularly suited for microbial community data.

For functional trait analysis, the microeco package was used to assign functional traits based on established databases (FAPROTAX for bacteria and FungalTraits for fungi) (Liu et al. 2021; Louca et al. 2016; Põlme et al. 2020). While FAPROTAX was developed for understanding oceanic microbiomes, it has also been shown effective for assessing soil bacteria (Sansupa et al. 2021). To enhance interpretability and reduce redundancy, only a subset of ecologically distinct functional traits was included in the analysis, focusing on those most relevant to soil ecosystem services such as nutrient cycling, organic matter decomposition, and pollutant degradation (Tables 5 & 6).

Afterwards, both the diversity and composition of selected functional traits and the diversity of taxa contributing to these traits were assessed.

To examine trait-level diversity, alpha diversity indices were calculated based on the relative read abundance of each selected trait, using Shannon diversity to evaluate trait richness and evenness. This approach provided insights into how functional diversity responded to different treatments, including mulch type, nitrogen addition, and land use. Beta diversity of functional traits was assessed using Bray-Curtis dissimilarity, followed by distance-based redundancy analysis (db-RDA) in the vegan package (Dixon and Palmer 2003; Oksanen et al. 2007), which allowed for a detailed assessment of trait composition differences between treatment groups. The db-RDA enabled evaluation of how mulch type, nitrogen application, and land use influenced the functional composition of microbial communities. To further understand the relationships between specific functional traits and environmental variables, the envfit function was used to fit environmental vectors to the ordination, identifying traits strongly associated with particular treatments and clarifying patterns within the db-RDA.

In addition to trait-level diversity, taxa-level diversity within each functional trait group was analyzed to provide deeper insights into the community composition underlying each function. For each selected functional trait, alpha diversity indices (Shannon diversity) were calculated to capture changes in richness and evenness of taxa contributing to each functional trait. General linear models were then used to assess how within-trait diversity differed across land use, mulch, and urea treatments (with interaction terms between treatments) with the Benjamini–Hochberg procedure applied to correct for multiple comparison.

Table 5. Selected bacteria functional traits examined as indicators of various ecosystems services and the interpretation of within-trait taxa diversity scores.

Trait (Bacteria/Fungi)	Ecosystem Service	Justification	Interpretation of Within-Trait Taxa Diversity
Ureolysis	Nutrient Cycling	Ureolysis contributes to nitrogen cycling by converting urea into ammonia, aiding soil fertility.	High diversity indicates resilience in nitrogen cycling processes, potentially enhancing adaptability to different nitrogen levels.
Nitrification	Nutrient Cycling	Nitrification facilitates the conversion of ammonia to nitrate, an essential step in nitrogen cycling, making nitrogen more readily available for plant uptake.	High diversity indicates a robust capacity for nitrogen transformation, promoting ecosystem resilience and maintaining soil fertility under varied conditions.
Nitrate Denitrification	Nutrient Cycling and Greenhouse Gas Regulation	Denitrification reduces nitrate to nitrogen gas (N_2), preventing nitrate leaching and nitrogen pollution. However, it can also produce nitrous oxide (N_2O), a potent greenhouse gas, as an intermediate product. Managing denitrification effectively can balance nutrient cycling benefits with minimizing GHG emissions.	High diversity suggests a stable denitrification capacity, which supports nutrient cycling while reducing risks of nitrate pollution. Diversity may also indicate varied efficiencies in nitrous oxide reduction, influencing overall GHG impacts. Nitrous oxide emissions depend largely on the continued availability of carbon and anoxic environments to allow complete rather than incomplete denitrification.
Nitrogen Fixation	Nutrient Cycling	Converts atmospheric nitrogen to ammonia, making nitrogen available to plants.	High diversity may support plant productivity by ensuring stable nitrogen availability.
Iron Respiration	Nutrient Cycling and Pollutant Remediation	Iron respiration contributes to the cycling of iron and associated nutrients, such as phosphorus and sulfur, and plays a role in breaking down organic pollutants. It also stabilizes soil structure and influences redox processes in anaerobic environments.	High diversity suggests greater resilience and efficiency in nutrient cycling and pollutant degradation, particularly in waterlogged or anaerobic soils.
Methanotrophy	Greenhouse Gas Regulation	Methanotrophy involves the oxidation of methane to CO_2, mitigating methane emissions and contributing to the reduction of greenhouse gases in the atmosphere.	Greater diversity enhances the capacity to oxidize methane under different soil conditions, supporting the regulation of greenhouse gas emissions and climate stability.
Chitinolysis	Decomposition	Decomposes chitin, contributing to the breakdown of organic matter in soil.	Diverse taxa enhance organic matter turnover and nutrient recycling, supporting soil health.
Cellulolysis	Decomposition	Breaks down cellulose, promoting decomposition and carbon cycling in soil.	A range of cellulolytic taxa can improve soil carbon cycling and organic matter quality.
Fermentation	Decomposition	Supports anaerobic breakdown of organic matter, important in low-oxygen environments.	Greater diversity suggests efficient organic matter breakdown under various conditions.
Aromatic Compound Degradation	Pollutant Degradation	Degrades complex aromatic compounds, helping reduce soil pollutants.	High taxa diversity may enhance resilience in pollutant degradation, supporting soil health.
Hydrocarbon Degradation	Pollutant Degradation	Breaks down hydrocarbons, aiding in soil remediation and reducing contamination.	Diverse hydrocarbon degraders enhance soil's ability to recover from contamination.
Human Pathogenic Capacity (All)	Health Indicators/Pathogen Protection	Indicates potential risks to human health through pathogen capacity in the soil.	Low diversity with low collective relative abundance in pathogenic taxa is desirable to minimize health risks in soil. Low diversity with high collective relative abundance could indicate the overgrowth and dominance of a single or few microbes with pathogenic capacity, potentially posing heightened risk.
Animal Parasites or Symbionts	Health Indicators	Reflects potential for animal pathogens or symbionts in soil, affecting biosecurity.	Controlled diversity could reduce risks of disease transmission to animals.

Table 6. Selected fungi functional traits examined as indicators of various ecosystems services and the interpretation of within-trait taxa diversity scores.

Soil Saprotroph (Primary Lifestyle)	Nutrient Cycling/Decomposition	Decomposes organic material, enhancing nutrient availability and soil structure.	High diversity in saprotrophs supports efficient organic matter breakdown and nutrient cycling.
Decay Substrate: Leaf/Fruit/Seed	Decomposition	Targets specific decaying substrates, contributing to organic matter turnover.	Diverse decomposers of various substrates promote overall soil health and decomposition rate.
Decay Type: Chitinolytic	Decomposition	Specializes in breaking down chitin, supporting nutrient recycling in soil.	Diverse chitinolytic taxa enhance decomposition of complex organic materials, aiding soil fertility.
Arbuscular Mycorrhizal (Primary Lifestyle)	Plant Health/Productivity	Forms symbiotic relationships with plants, improving nutrient and water uptake.	High diversity supports plant resilience and productivity by providing varied nutrient support.
Ectomycorrhizal (Primary Lifestyle)	Plant Health/Productivity	Enhances phosphorus uptake for host plants, benefiting tree and shrub health.	Diverse ectomycorrhizal taxa strengthen plant community resilience, especially in forests.
Root Endophyte (Primary Lifestyle)	Plant Health/Productivity	Lives within roots, providing benefits like disease resistance and growth promotion.	High diversity may improve plant health by offering multiple forms of protection.
Root-Associated Endophyte Interaction	Plant Health/Productivity	Provides plant roots with enhanced resilience against pathogens and nutrient uptake.	A range of taxa strengthens plant resistance to environmental stresses.
Leaf/Fruit/Seed Pathogen (Plant Pathogenic Capacity)	Plant Pathogens	Indicates potential plant disease risk in soil, affecting agricultural productivity.	Low diversity is desirable to limit potential pathogenic impact on crops.
Root Pathogen (Plant Pathogenic Capacity)	Plant Pathogens	Reflects risk of root diseases that could reduce plant growth and yield.	Controlled diversity minimizes root disease risk, promoting healthier crop systems.
Wood Pathogen (Plant Pathogenic Capacity)	Plant Pathogens	Affects woody plants, posing risks for forest health and timber productivity.	Lower diversity could reduce disease spread, benefiting forest and timber health.
Nematophagous (Animal Biotrophic Capacity)	Pathogen Suppression/Biosecurity	Acts as a biological control against nematodes, contributing to biosecurity and crop health.	High diversity supports pest suppression, potentially reducing the need for chemical nematicides.
Filamentous Mycelium (Growth Form)	Water Regulation/Environmental Resilience	Contributes to soil structure and moisture retention.	High diversity strengthens soil stability and resilience to water stress.
Partly Aquatic (Aquatic Habitat)	Environmental Resilience	Adapts to fluctuating water levels, supporting resilience in variable moisture environments.	Greater diversity supports adaptation to variable moisture conditions, aiding soil health.
Opportunistic Human Parasite (Animal Biotrophic Capacity)	Human Health Indicators	Reflects potential human health risks in soil, important for biosecurity.	Low diversity reduces potential health risks from human-associated pathogens.
Foliar Endophyte (Primary Lifestyle)	Human Health Indicators	Interacts with plant leaves, potentially affecting plant health and human exposure.	Controlled diversity limits health risks while supporting plant resilience.

5.3 RESULTS

5.3.1 SOIL PHYSICOCHEMISTRY

The Redundancy Analysis (RDA) results showed that 56.6% of the total variance in soil physicochemical properties was explained by the constrained variables (Veg, Mulch, Urea, and Mulch*Urea interaction), while 43.4% of the variance remained unconstrained (Figure 1). The first two RDA axes captured the majority of this explained variance, with RDA1 accounting for 49.3% and RDA2 contributing 5.5%, indicating strong treatment effects.

ANOVA testing of the constrained variables revealed significant effects for both Veg ($F = 15.36$, $df = 2$, $p < 0.001$) and Mulch ($F = 37.36$, $df = 2$, $p < 0.001$) on soil properties, while Urea ($F = 0.42$, $df = 1$, $p = 0.578$) and the Mulch*Urea interaction ($F = 0.48$, $df = 2$, $p = 0.647$) were not significant. The environmental fit analysis (envfit) identified five variables strongly associated with the RDA axes: Phosphorus Buffer Index, Potassium (Amm. Acet.), Magnesium (Amm. Acet.), Cation Exchange Capacity (CEC incl. Al), and Available Potassium, each with high correlation values ($r > 0.8$) and significant p-values (Figure 2; $p < 0.001$).

Hierarchical clustering grouped these variables into two clusters based on similarity in their responses to treatments. Cluster 1 included Phosphorus Buffer Index, Calcium, Magnesium, and CEC, which are linked to soil buffering and nutrient-holding capacity. Cluster 2 contained Potassium and Available Potassium, indicating a strong role in immediate nutrient availability. These clusters simplify interpretation by highlighting patterns in soil chemistry across the experimental treatments.

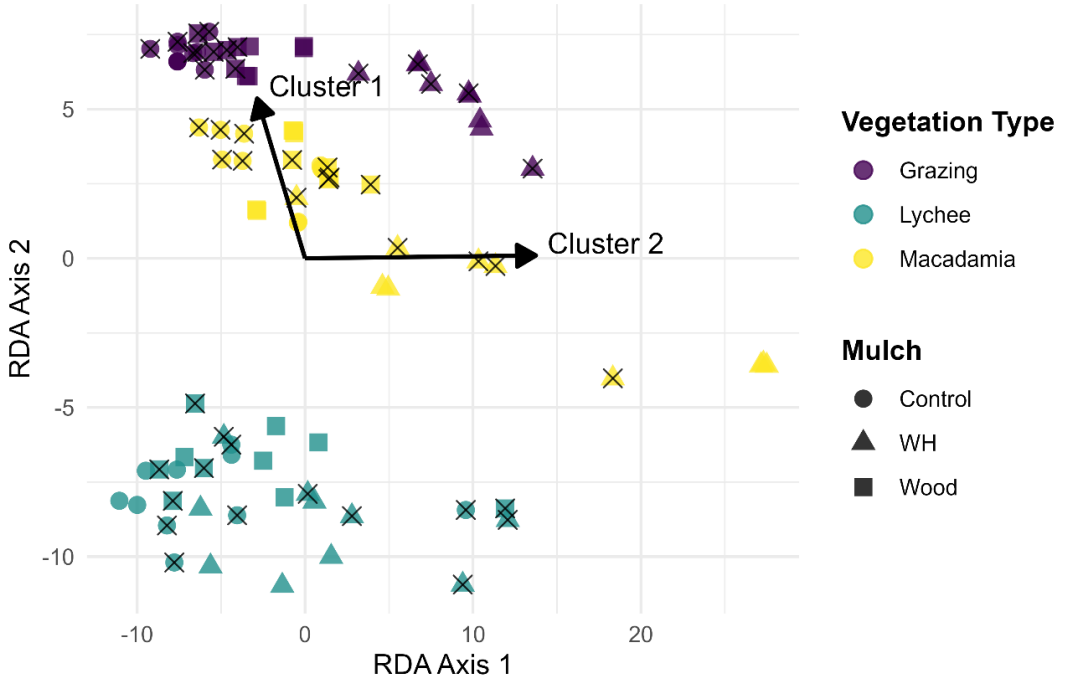

Figure 1. Redundancy Analysis (RDA) plot showing soil physicochemical parameters across experimental treatments. Points represent individual soil samples, with colours indicating vegetation type (Veg), shapes indicating mulch treatment (Mulch), and crosses representing urea-treated samples. Arrows indicate significant soil parameters ($p < 0.05$) correlated with the RDA axes, scaled by correlation strength. Key parameters cluster into two groups: Cluster 1 (e.g., Phosphorus Buffer Index, Calcium, Magnesium, and CEC) associated with soil buffering and nutrient-holding capacity, and Cluster 2 (Potassium) related to nutrient availability. Axis scaling reflects the range of treatment effects on soil properties.

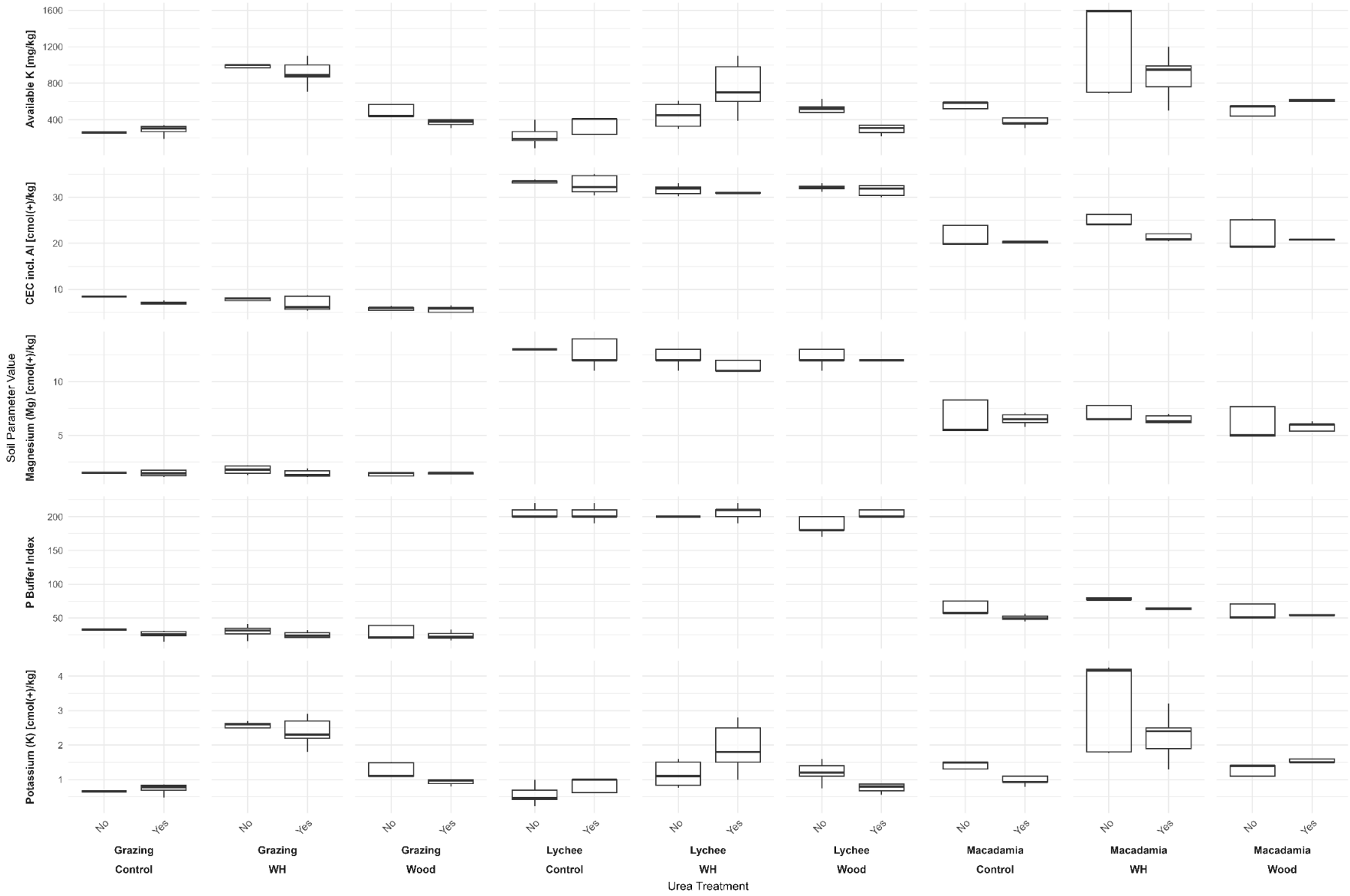

Figure 2. Boxplots of selected soil parameters for each treatment combination of vegetation type (Veg), mulch type (Mulch), and urea application (Urea).

5.3.1 BACTERIA ASSEMBLAGES

An ANOVA on Shannon diversity revealed a significant effect of vegetation type (Veg) on bacterial diversity ($F = 17.66$, $df = 2$, $p < 0.001$), indicating that vegetation type strongly influences bacterial community richness and evenness. Neither mulch type nor urea application had significant main effects on diversity, nor did their interaction terms contribute meaningfully to explaining variability.

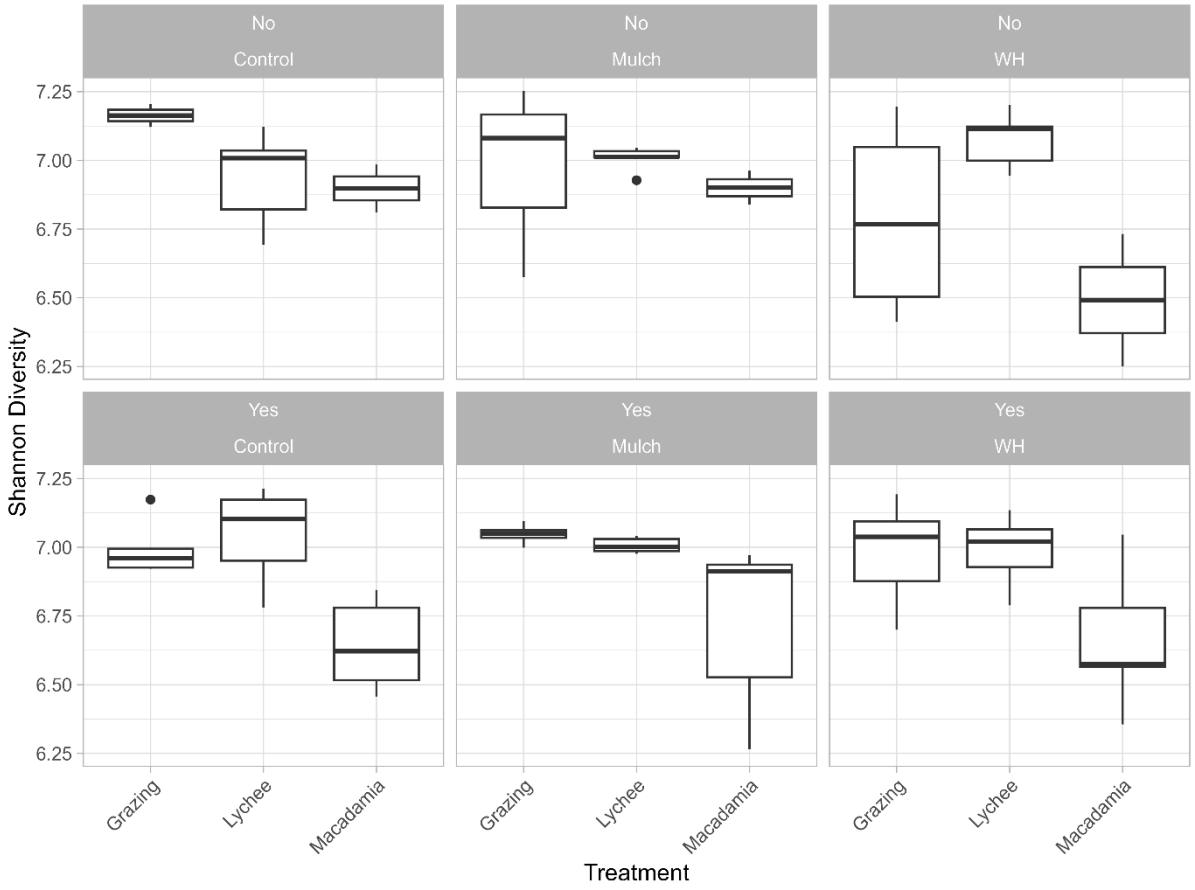

Figure 3. The Shannon's diversity of bacteria ASVs across treatments. Yes and No indicate whether urea was applied. WH indicates water hyacinth.

A distance-based redundancy analysis (db-RDA) and an ANOVA-like permutation test (999 permutations) assessed how vegetation type, mulch, and urea treatments influenced bacterial community composition based on Bray-Curtis dissimilarity (Figure 4). Vegetation type had the strongest effect ($F = 27.59$, $df = 2$, $p < 0.001$), highlighting its pivotal role in shaping community composition. Both mulch ($F = 1.94$, $df = 2$, $p = 0.015$) and urea ($F = 2.40$, $df = 1$, $p = 0.015$) also significantly influenced beta diversity. The interaction between mulch and urea was not significant ($p = 0.31$), indicating these factors likely have additive rather than interactive effects.

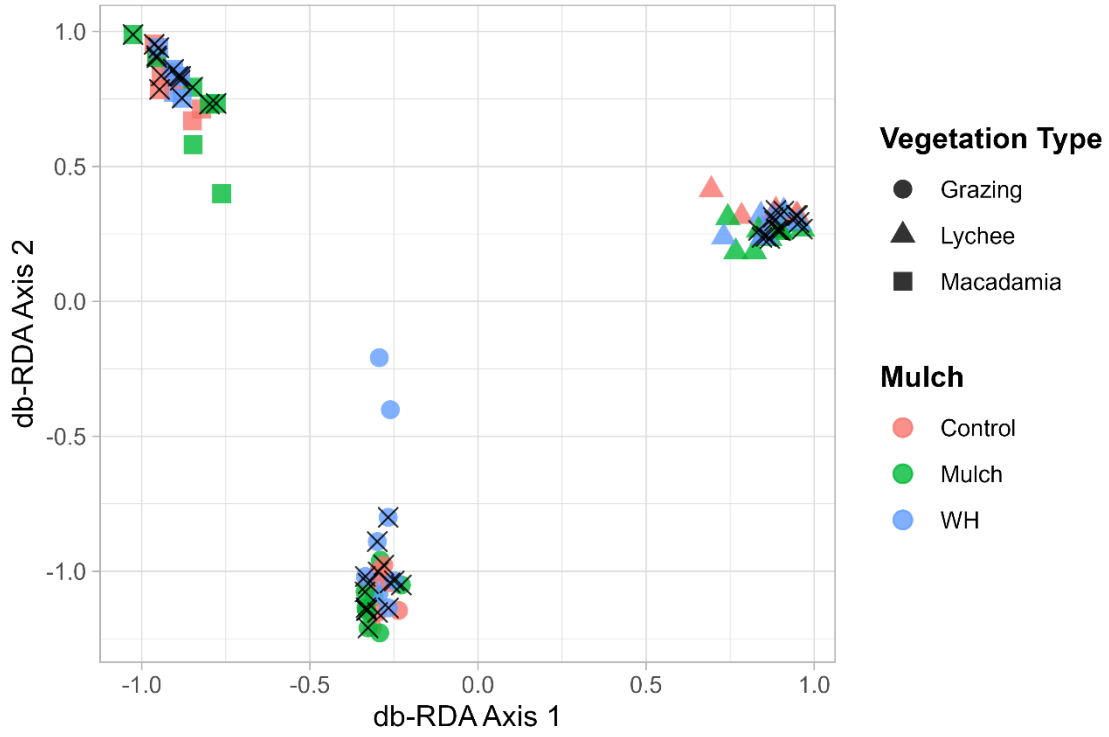

Figure 4. Redundancy Analysis (RDA) plot showing soil bacteria genera assemblages across experimental treatments. Points represent individual soil samples, with shapes indicating vegetation type (Veg), colours indicating mulch treatment (Mulch), and crosses representing urea-treated samples.

Differential abundance analysis of bacterial communities revealed significant shifts in specific taxa in response to mulch type, urea addition, and vegetation type (Figure 5). The largest changes in bacterial families were observed across treatments involving mulch type, urea addition, and vegetation type. Pseudomonadaceae (Proteobacteria) exhibited significant increases under macadamia vegetation compared to grazing (log2FoldChange = 6.06) and water hyacinth mulch compared to control (log2FoldChange = 4.88). In contrast, Pseudomonadaceae decreased substantially under water hyacinth mulch in lychee compared to grazing (log2FoldChange = -5.18). Similarly, Chthoniobacteraceae (Verrucomicrobia) showed marked declines under lychee vegetation compared to grazing (log2FoldChange = -3.95) but increased with water hyacinth mulch in grazing (log2FoldChange = 1.35). Nitrosomonadaceae (Proteobacteria) decreased under wood mulch in macadamia compared to control (log2FoldChange = -2.50) but increased with water hyacinth mulch in grazing (log2FoldChange = 1.73).

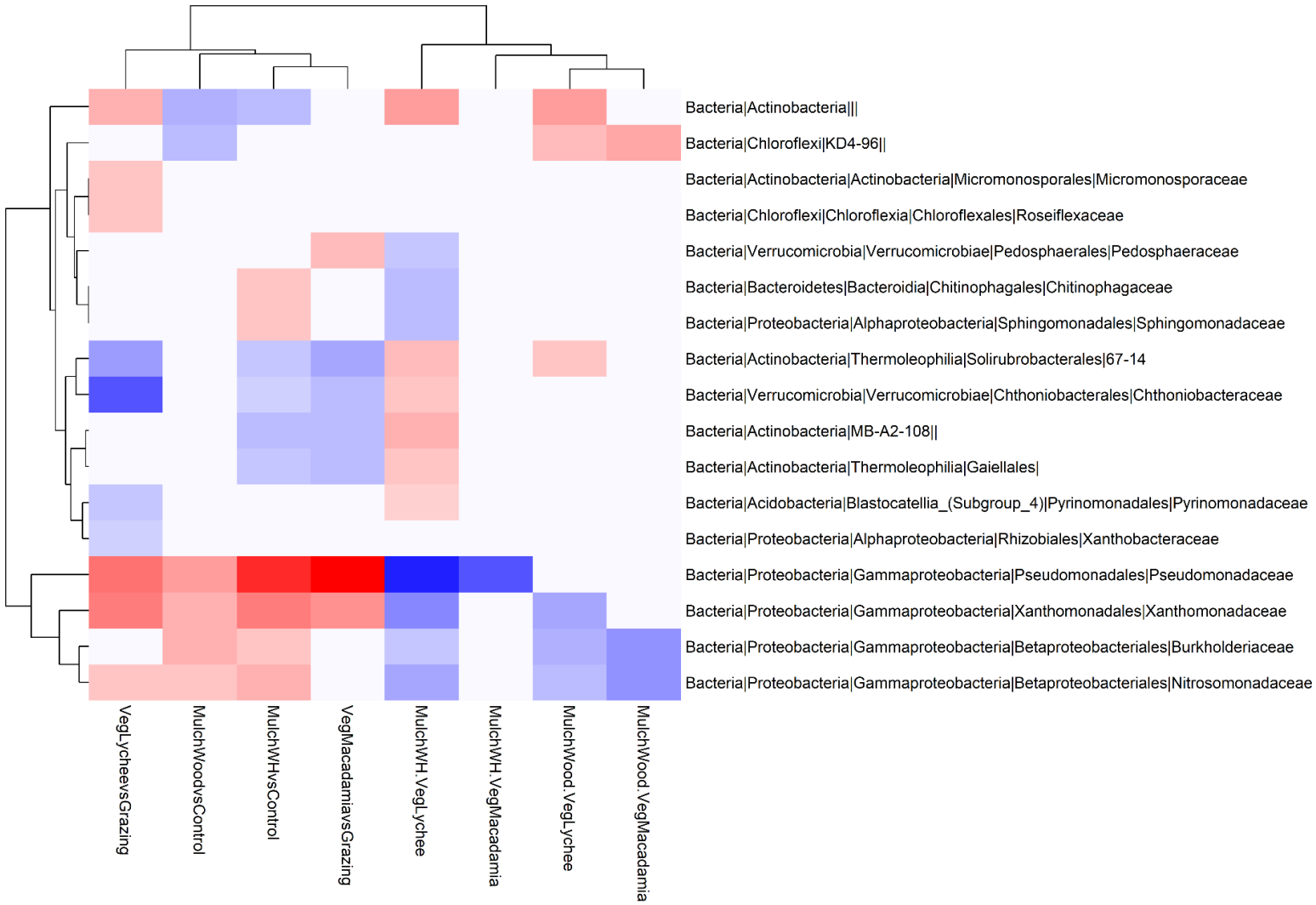

Figure 5. Heatmap showing the $\log_2$ fold changes of bacterial families across treatment combinations of vegetation type (Macadamia, Lychee, or Grazing), mulch type (Water Hyacinth, Wood, or Control), and urea application (Yes or No), relative to the baseline scenario of grazing without mulch or urea. Differential abundance was determined using DESeq2, with only significant taxa (adjusted p-value < 0.05) included in the plot. Blue represents decreases, red represents increases, and white indicates no significant change. Taxa and treatments are hierarchically clustered using Euclidean distance and complete linkage, with treatments ordered to group comparisons within the same vegetation type for easier interpretation.

A distance-based redundancy analysis (dbRDA) was conducted to assess the relationships between bacterial functional traits (e.g., nitrification, chitinolysis, and fermentation) and the explanatory variables vegetation type, mulch type, and urea addition (Figure 6). The analysis explained 34.3% of the variation in bacterial functional traits, with constrained axes contributing 0.5224 inertia and unconstrained axes contributing 0.9997 inertia. Among the constrained axes, dbRDA1 and dbRDA2 captured the majority of the explained variance, with eigenvalues of 0.28371 and 0.17571, respectively.

The permutation test of marginal effects revealed that vegetation type was the strongest predictor ($F = 14.61$, $p < 0.001$), followed by mulch type ($F = 3.30$, $p = 0.002$), while urea addition was not significant ($F = 1.38$, $p = 0.231$). Best-fit vectors indicated that nitrification ($r^2 = 0.92$, $p = 0.001$), fermentation ($r^2 = 0.54$, $p = 0.001$), and chitinolysis ($r^2 = 0.53$, $p = 0.001$) were the most strongly correlated functional traits with the constrained axes. Nitrification was negatively associated with dbRDA1, while fermentation and chitinolysis were positively associated with dbRDA2.

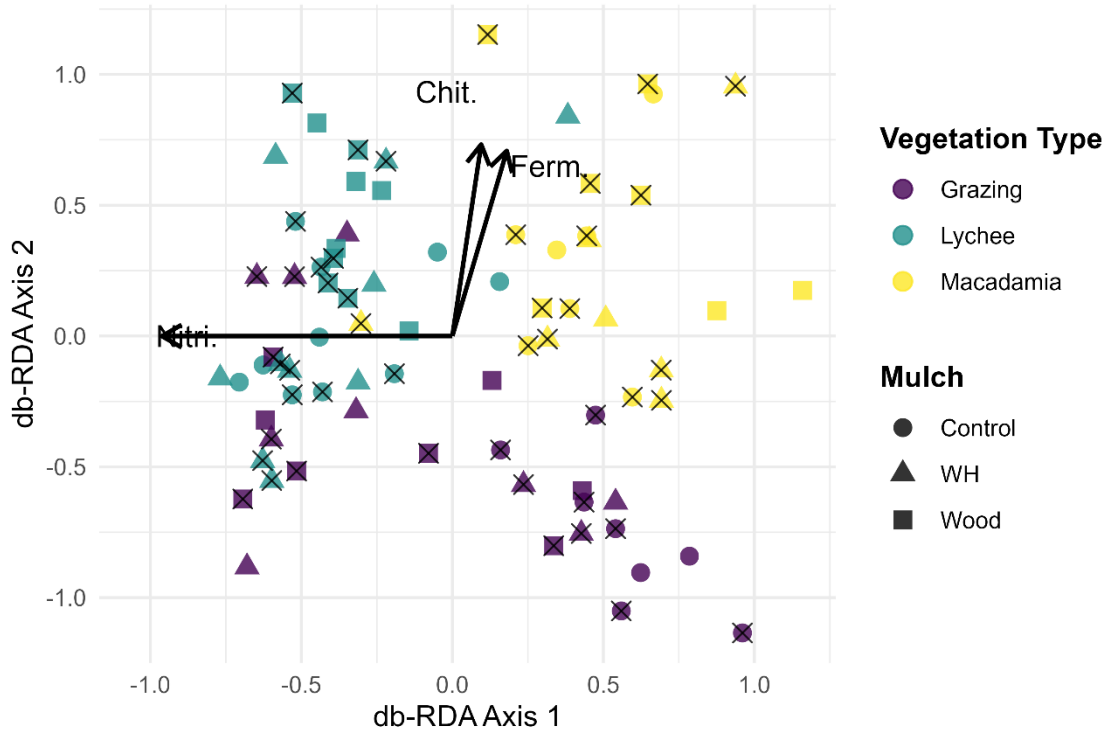

Figure 6. Distance-based redundancy analysis (dbRDA) plot showing the relationships between bacterial functional traits and explanatory variables (vegetation type, mulch type, and urea addition). The dbRDA analysis explains 34.3% of the variation in functional traits, with dbRDA1 and dbRDA2 capturing the majority of the constrained variance. Key functional traits, including nitrification, fermentation, and chitinolysis, are represented as vectors indicating their correlation with the constrained axes. Vegetation type and mulch type significantly influence the distribution of bacterial functional traits ($p < 0.001$ and $p = 0.002$, respectively), while urea addition shows no significant effect ($p = 0.231$). Longer vectors indicate stronger correlations with the axes.

The analysis of within-trait taxa Shannon diversity identified several significant predictors affecting various ecosystem service traits (Figure 7). Nitrate denitrification was significantly influenced by the interaction of vegetation type, mulch type, and urea application, with the combination of VegLychee:MulchWood increasing diversity (Estimate = 0.89, adj. p = 0.015) and VegLychee:MulchWood:UreaYes decreasing it (Estimate = -0.99, adj. p = 0.004). Nitrogen fixation was positively associated with VegLychee:MulchWood (Estimate = 0.48, adj. p = 0.030), while chitinolysis showed increased diversity under VegLychee and VegMacadamia (Estimates = 0.80 and 0.96, adj. p = 0.028 and 0.004, respectively). For methanotrophy, significant interactions included MulchWH (Estimate = 0.14, adj. p < 0.001) and various combinations involving vegetation, mulch, and urea, with distinct positive and negative effects depending on the treatment (e.g., VegMacadamia:MulchWH = 0.39, adj. p < 0.001; MulchWH:UreaYes = -0.14, adj. p < 0.001). Lastly, animal parasites or symbionts were notably influenced by vegetation, mulch, and urea interactions, with VegMacadamia combined with MulchWood and UreaYes yielding the strongest positive effect (Estimate = 3.64, adj. p < 0.001), while other interactions such as VegMacadamia:MulchWood without urea reduced diversity (Estimate = -3.06, adj. p < 0.001).

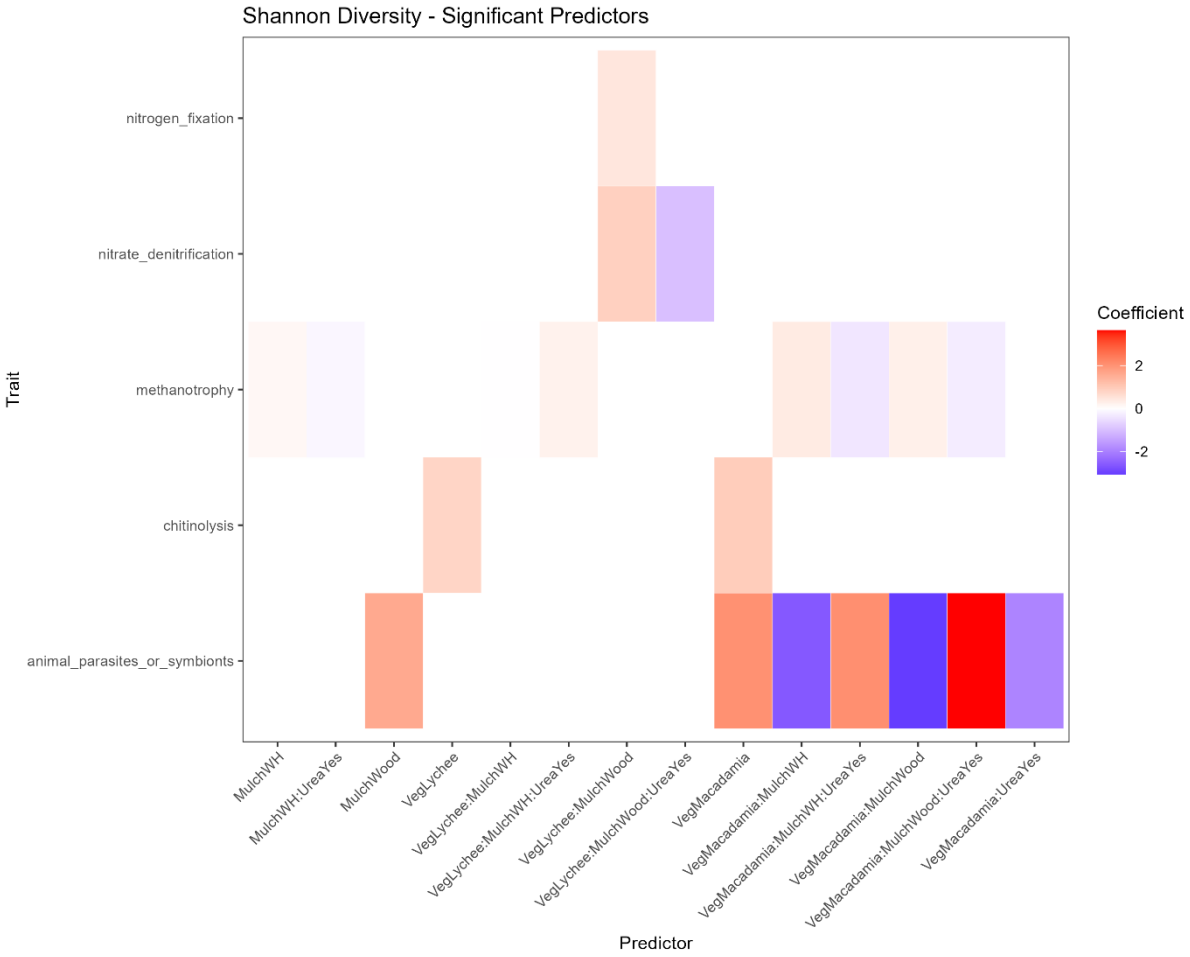

Figure 7. Heatmap showing significant predictors of within-taxa Shannon diversity for bacteria functional traits. Rows represent traits, and columns represent experimental treatments predictors, including vegetation type (Veg), mulch type (Mulch), and urea application (Urea) with their interactions. Colour intensity indicates the magnitude and direction of the coefficients, with red representing positive associations and blue representing negative associations. Comparisons are against a grazing, no mulch, and no urea baseline. Only significant predictors (adjusted p-value < 0.05) are shown.

5.3.2 FUNGAL ASSEMBLAGES

An ANOVA on Shannon diversity revealed a significant effect of vegetation type (Veg) on fungal diversity ($F = 5.48$, $df = 2$, $p < 0.001$), indicating that vegetation type strongly influences fungal community richness and evenness (Figure 8). Neither mulch type nor urea application had significant main effects on diversity, nor did their interaction terms contribute meaningfully to explaining variability.

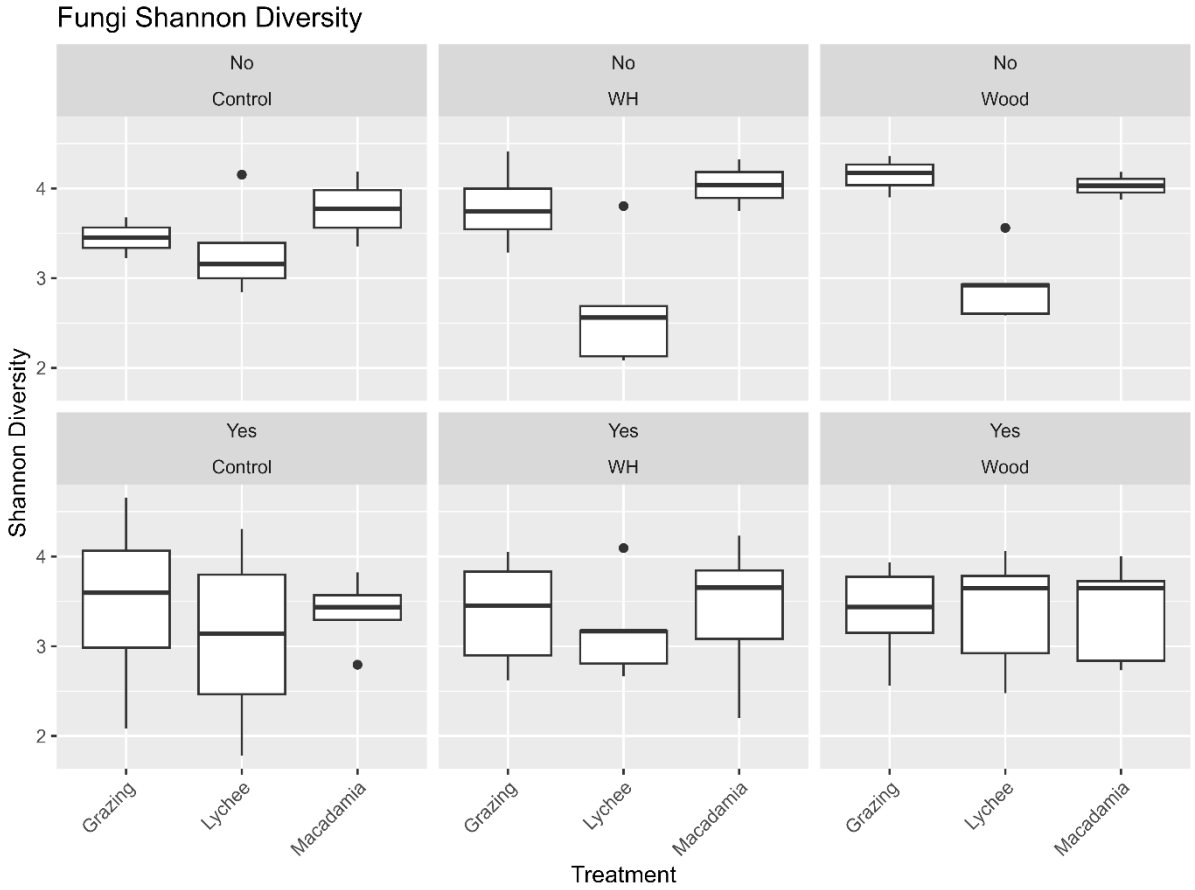

Figure 8. The Shannon's diversity of fungi ASVs across treatments. Yes and No indicate whether urea was applied. WH indicates water hyacinth.

A distance-based redundancy analysis (db-RDA) and an ANOVA-like permutation test (999 permutations) assessed how vegetation type, mulch, and urea treatments influenced fungal community composition based on Bray-Curtis dissimilarity. Vegetation type had the strongest effect (Figure 9; $F = 19.66$, $df = 2$, $p < 0.001$), highlighting its pivotal role in shaping community composition. Mulch had marginal significance ($F = 1.43$, $df = 2$, $p = 0.085$), while urea was not significantly influential on beta diversity ($F = 1.52$, $df = 1$, $p = 0.119$).

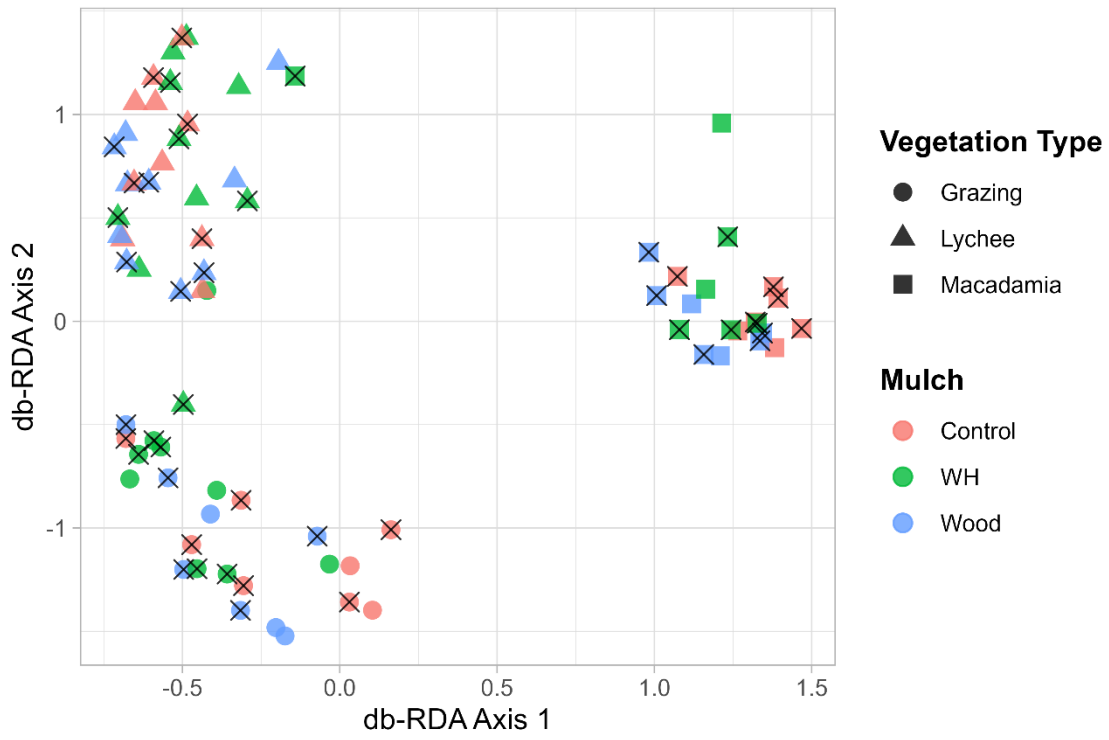

Figure 9. Redundancy Analysis (RDA) plot showing soil fungi genera assemblages across experimental treatments. Points represent individual soil samples, with shapes indicating vegetation type (Veg), colours indicating mulch treatment (Mulch), and crosses representing urea-treated samples.

Differential abundance analysis of fungal communities using DESeq2 revealed significant differences in specific fungal taxa across combinations of vegetation type, mulch, and urea addition (Figure 10). The most pronounced differences were observed under macadamia and lychee vegetation types compared to grazing, and in treatments involving water hyacinth mulch. Leotiomycetes (Ascomycota) exhibited significantly greater abundance under macadamia vegetation with water hyacinth mulch and urea addition (log2FoldChange = 5.37) and under lychee compared to grazing (log2FoldChange = 3.43), while their abundance was lower under lychee with urea addition (log2FoldChange = -3.39). Sordariomycetes (Ascomycota) showed greater abundance under macadamia vegetation compared to grazing (log2FoldChange = 2.64), but lower abundance under water hyacinth mulch with urea addition (log2FoldChange = -2.03). Dothideomycetes (Ascomycota) were more abundant under macadamia vegetation with water hyacinth mulch and urea addition (log2FoldChange = 3.58), but their abundance was lower in the same mulch-urea treatment under lychee (log2FoldChange = -2.59). Mucoromycotina_X (Mucoromycota) exhibited greater abundance under water hyacinth mulch compared to control (log2FoldChange = 3.40) but lower abundance with the addition of urea (log2FoldChange = -3.53). Lastly, Agaricomycetes (Basidiomycota) showed greater abundance under water hyacinth mulch and urea addition (log2FoldChange = 3.01), but lower abundance in macadamia vegetation compared to grazing (log2FoldChange = -3.68).

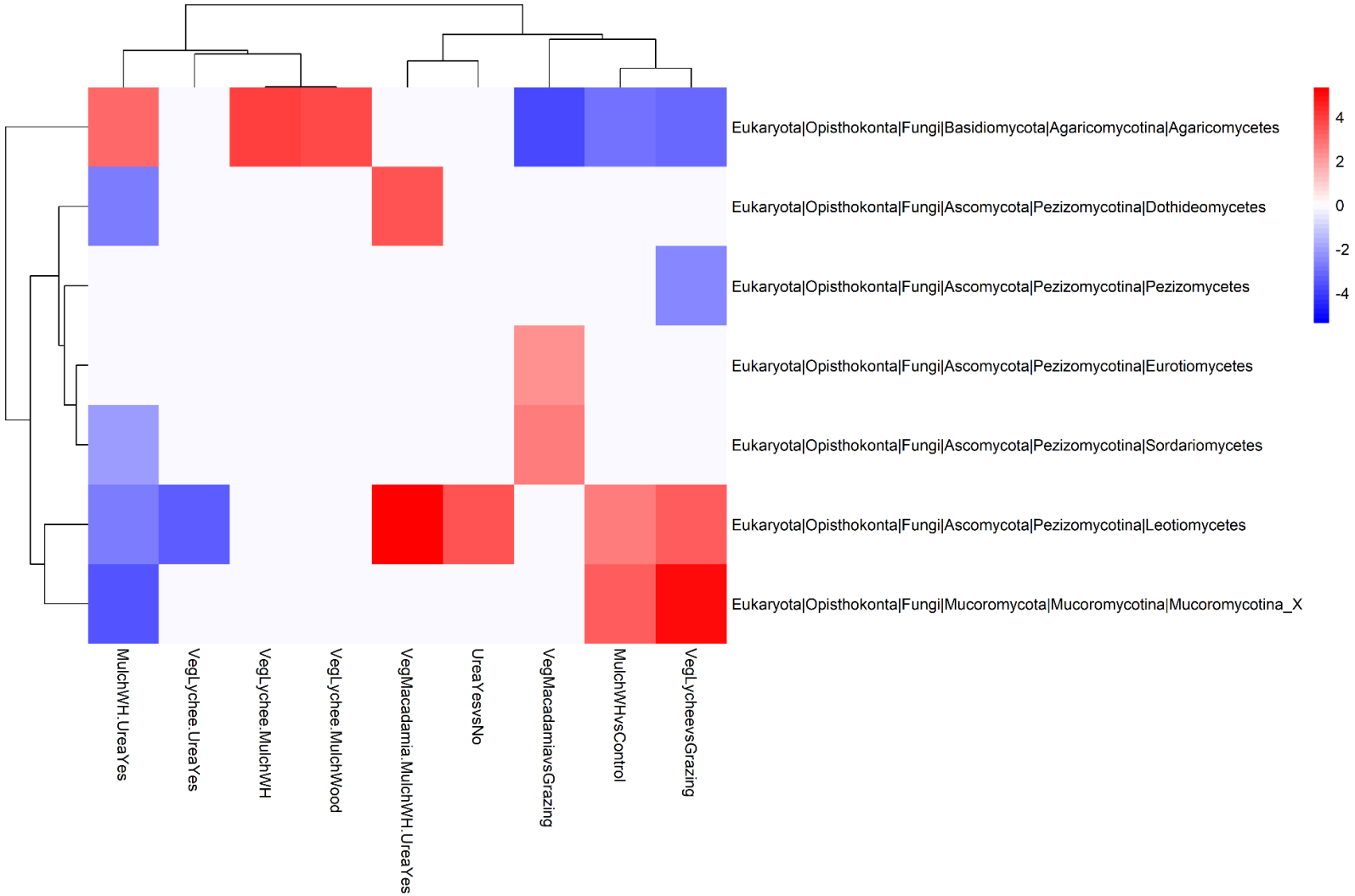

Figure 10. Heatmap showing the $\log_2$ fold changes of fungal families across treatment combinations of vegetation type (Macadamia, Lychee, or Grazing), mulch type (Water Hyacinth, Wood, or Control), and urea application (Yes or No), relative to the baseline scenario of grazing without mulch or urea. Differential abundance was determined using DESeq2, with only significant taxa (adjusted p-value < 0.05) included in the plot. Blue represents decreases, red represents increases, and white indicates no significant change. Taxa and treatments are hierarchically clustered using Euclidean distance and complete linkage, with treatments ordered to group comparisons within the same vegetation type for easier interpretation.

A distance-based redundancy analysis (dbRDA) was conducted to explore the relationships between fungal functional traits (e.g., soil saprotrophy, chitinolysis, and leaf/fruit decay) and the explanatory variables vegetation type, mulch type, and urea addition (Figure 11). The analysis accounted for 49.6% of the variation in fungal functional traits, with constrained axes contributing 3.433 inertia and unconstrained axes contributing 3.483 inertia. Among the constrained axes, dbRDA1 and dbRDA2 explained the majority of the variance, with eigenvalues of 2.6321 (76.7%) and 0.7307 (21.3%), respectively. A permutation test revealed that vegetation type was the strongest predictor ($F = 28.60$, $p < 0.001$), while neither mulch type ($F = 1.64$, $p = 0.15$) nor urea addition ($F = 1.71$, $p = 0.153$) were significant.

Best-fit vectors highlighted strong correlations of soil saprotrophy ($r^2 = 0.86$, $p = 0.001$), chitinolysis ($r^2 = 0.87$, $p = 0.001$), and leaf/fruit decay ($r^2 = 0.87$, $p = 0.001$) with the constrained axes. Soil saprotrophy and chitinolysis were positively associated with dbRDA2, while leaf/fruit decay was negatively associated with dbRDA1.

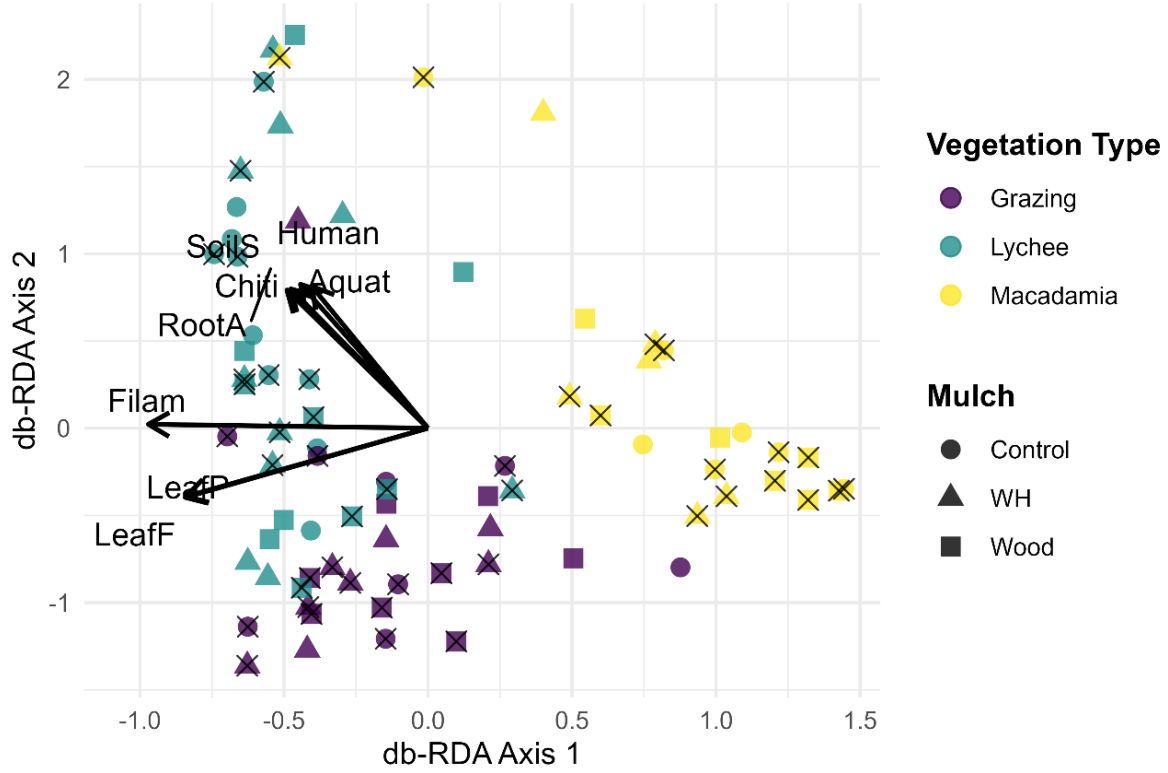

Figure 11. Distance-based redundancy analysis (dbRDA) plot showing the relationships between fungal functional traits and explanatory variables (vegetation type, mulch type, and urea addition). The abbreviations represent key fungal traits: "SoilS" for soil saprotrophs, "LeafF" for decomposition of leaf, fruit, or seed substrates, "Chiti" for chitinolytic activity, "RootA" for root-associated endophytes, "LeafP" for leaf, fruit, or seed pathogens, "Filam" for filamentous mycelium, "Aquat" for partly aquatic fungi, and "Human" for opportunistic human parasites.

The within-trait taxa diversity analysis revealed significant relationships between traits and environmental predictors (Figure 12). Soil saprotroph diversity increased under lychee vegetation, wood mulch, and urea application (β = 0.881, p = 0.034), while taxa involved in leaf, fruit, and seed decomposition were more diverse under macadamia vegetation (β = 1.166, p = 0.044). Chitinolytic diversity increased with macadamia vegetation, water hyacinth mulch, and urea application (β = 0.602, p = 0.037). Arbuscular mycorrhizal diversity increased with water hyacinth mulch (β = 0.997, p = 0.005), wood mulch (β = 0.840, p = 0.025), and urea application (β = 0.995, p = 0.005), but decreased under lychee vegetation with water hyacinth mulch (β = -1.178, p < 0.001) or wood mulch (β = -1.011, p = 0.005). Ectomycorrhizal diversity was lower under lychee (β = -0.856, p = 0.037) and macadamia vegetation (β = -0.856, p = 0.037), but increased with macadamia vegetation and wood mulch (β = 0.969, p = 0.014). Wood pathogen diversity decreased with macadamia vegetation and wood mulch (β = -1.235, p = 0.037). Nemathophagous diversity increased with wood mulch (β = 0.932, p = 0.039) but decreased under macadamia vegetation and wood mulch (β = -0.903, p = 0.046). Filamentous mycelium diversity increased with lychee vegetation, wood mulch, and urea (β = 1.035, p = 0.049). Opportunistic human parasite diversity increased with macadamia vegetation (β = 0.575, p < 0.001), water hyacinth mulch (β = 0.339, p = 0.005), and wood mulch (β = 0.308, p = 0.012), but decreased under lychee vegetation with water hyacinth mulch (β = -0.918, p < 0.001) or wood mulch (β = -0.687, p < 0.001).

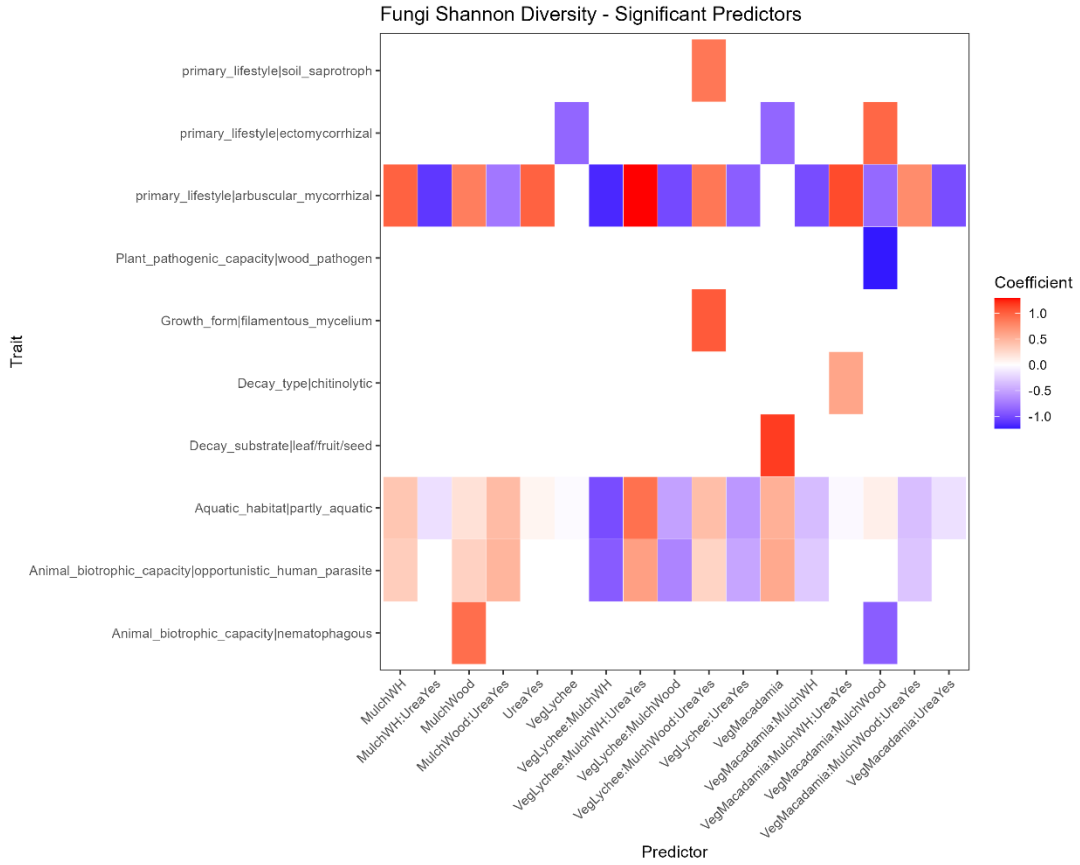

Figure 12. Heatmap showing significant predictors of within-taxa Shannon diversity for fungal functional traits. Rows represent traits, and columns represent experimental treatments predictors, including vegetation type (Veg), mulch type (Mulch), and urea application (Urea) with their interactions. Colour intensity indicates the magnitude and direction of the coefficients, with red representing positive associations and blue representing negative associations. Comparisons are against a grazing, no mulch, and no urea baseline. Only significant predictors (adjusted p-value < 0.05) are shown.

5.4 DISCUSSION

This study examined the effects of mulch type, nitrogen addition, and vegetation type on soil microbial communities and their functional traits across macadamia, lychee, and grazing systems. The analysis focused on microbial diversity, community composition, and functional traits associated with ecosystem services, including nutrient cycling, decomposition, and pathogen suppression.

Vegetation type was a major driver of microbial diversity and functional traits, reflecting the influence of plant inputs and root-microbe interactions. For example, macadamia vegetation strongly supported traits such as leaf/fruit decomposition and soil saprotrophy, likely due to the high-quality organic inputs and specific root-associated communities in these systems. These findings align with studies showing that vegetation type modulates the quantity and quality of root exudates and litter inputs, shaping microbial community structure and function (Doornbos et al. 2012; Vives-Peris et al. 2020; Wu et al. 2018). In contrast, lychee vegetation was associated with declines in ectomycorrhizal diversity and shifts in decomposition traits, suggesting that competitive interactions or practices used to grow Lychee (such as large soil mounding) may reduce the abundance of specific fungal taxa.

The type of mulch had significant effects on microbial functional traits, particularly those associated with nutrient cycling and decomposition. Water hyacinth (WH) mulch increased the diversity of arbuscular mycorrhizal fungi, chitinolytic microbes, and other traits related to rapid nutrient cycling and decomposition. These results align with previous findings that labile organic matter inputs, such as those provided by WH mulch, stimulate microbial activity by increasing the availability of easily degradable carbon and nutrients (de Graaff et al. 2010; Derrien et al. 2014; Paterson and Sim 2013). However, this effect was context-dependent. For example, arbuscular mycorrhizal diversity decreased under WH mulch in lychee orchards, potentially due to specific interactions between WH mulch inputs and lychee root exudates or lychee agricultural practices. Woody mulch, in contrast, favoured traits associated with longer-term organic matter stabilization and soil structure, such as soil saprotrophy and nematophagous activity. These findings are consistent with the slower decomposition rates of woody material, which promote gradual carbon release and microbial colonization (Averill and Waring 2018; Lustenhouwer et al. 2020; Weedon et al. 2009). However, the increased diversity of opportunistic human pathogens under woody mulch highlights the need to monitor biosecurity risks associated with organic amendments (Banerjee and van der Heijden 2023; van Bruggen et al. 2019; Yan et al. 2022).

Nitrogen addition via urea had notable effects on microbial traits, often interacting with mulch and vegetation treatments. For example, urea increased the diversity of arbuscular mycorrhizal fungi, suggesting that added nitrogen may alleviate nutrient limitations for these mutualists, thereby enhancing their ability to support plant nutrient uptake. Similarly, chitinolytic diversity was elevated under macadamia vegetation and WH mulch with urea addition, indicating potential synergies between nitrogen availability, labile carbon inputs, and pathogen suppression traits. However, the negative interactions observed between urea addition and specific mulch treatments, such as reductions in methanotrophic and nematophagous diversity under WH mulch, highlight the potential trade-offs of nitrogen inputs. Excess nitrogen may shift microbial community composition, favouring taxa adapted to high nitrogen environments at the expense of others, potentially disrupting ecosystem functions.

The diversity of specific microbial traits provides insights into the functional resilience and ecosystem services of agricultural soils. For example, the increase in chitinolytic and saprotrophic diversity under WH mulch suggests enhanced decomposition and nutrient cycling, which could

improve soil fertility and crop productivity. Conversely, the reduced diversity of ectomycorrhizal fungi in lychee orchards highlights potential challenges in maintaining symbiotic relationships critical for phosphorus uptake and plant resilience. The observed increases in nematophagous diversity under woody mulch suggest potential for biological control of nematode pests, reducing the need for chemical nematicides. However, the elevated diversity of opportunistic human pathogens under certain treatments emphasizes the need for careful management to balance soil health benefits with biosecurity concerns.

This study has several limitations that should be considered. First, the experimental duration was relatively short, limiting the ability to observe long-term effects of mulch and urea treatments on soil microbial communities and functional traits. The use of a single application of urea and mulch may not capture the cumulative effects of repeated treatments often used in agricultural practices. Additionally, while metabarcoding provided detailed insights into microbial taxa and traits, it does not account for functional redundancy or metabolic activity, which may influence ecosystem processes. Finally, the study was conducted in three specific agricultural settings, which may limit the generalizability of findings to other land uses, soil types, or climatic conditions.

Future research should focus on addressing these limitations by conducting long-term experiments to evaluate the persistence of treatment effects and their potential cumulative impacts. Including functional assays alongside metabarcoding would provide a more comprehensive understanding of microbial activity and its contribution to ecosystem services. Expanding the study to additional land use types, soil conditions, and climatic regions would enhance the generalizability of findings. Furthermore, future studies should explore the interaction of mulch and urea treatments with other soil management practices, such as cover cropping or irrigation, to identify synergies that enhance soil health and productivity.

6. REFERENCES

Averill, C., and Waring, B. 2018. Nitrogen limitation of decomposition and decay: How can it occur? Global Change Biology **24**(4): 1417-1427. doi:https://doi.org/10.1111/gcb.13980.

Banerjee, S., and van der Heijden, M.G.A. 2023. Soil microbiomes and one health. Nature Reviews Microbiology **21**(1): 6-20. doi:10.1038/s41579-022-00779-w.

Bao, X., Li, M.Q., Niu, R.J., Lu, J.L., Panigrahi, S., Garg, A., and Berretta, C. 2021. Hygroscopic Water Retention and Physio-Chemical Properties of Three In-House Produced Biochars from Different Feedstock Types: Implications on Substrate Amendment in Green Infrastructure. WATER **13**(19). doi:10.3390/w13192613.

Bordoloi, S., Garg, A., Sreedeep, S., Lin, P., and Mei, G. 2018. Investigation of cracking and water availability of soil-biochar composite synthesized from invasive weed water hyacinth. Bioresource Technology **263**: 665-677. doi:https://doi.org/10.1016/j.biortech.2018.05.011.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 2016 13:7 **13**(7): 581-583. doi:10.1038/nmeth.3869.

de Graaff, M.-A., Classen, A.T., Castro, H.F., and Schadt, C.W. 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytologist **188**(4): 1055-1064. doi:https://doi.org/10.1111/j.1469-8137.2010.03427.x.

Derrien, D., Plain, C., Courty, P.-E., Gelhaye, L., Moerdijk-Poortvliet, T.C.W., Thomas, F., Versini, A., Zeller, B., Koutika, L.-S., Boschker, H.T.S., and Epron, D. 2014. Does the addition of labile substrate destabilise old soil organic matter? Soil Biology and Biochemistry **76**: 149-160. doi:https://doi.org/10.1016/j.soilbio.2014.04.030.

Dixon, P., and Palmer, M.W. 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science **14**(6): 927-930. doi:10.1658/1100-9233(2003)014[0927:VAPORF]2.0.CO;2.

Doornbos, R.F., van Loon, L.C., and Bakker, P.A.H.M. 2012. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agronomy for Sustainable Development **32**(1): 227-243. doi:10.1007/s13593-011-0028-y.

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D., Friendly, M., Gorjanc, G., and Graves, S. 2012. Package 'car'. Vienna: R Foundation for Statistical Computing **16**(332): 333.

Gezahegn, A., Selassie, Y.G., Agegnehu, G., Addisu, S., Mihretie, F.A., Kohira, Y., and Sato, S. 2024. Pyrolysis temperature changes the physicochemical characteristics of water hyacinth-based biochar as a potential soil amendment. BIOMASS CONVERSION AND BIOREFINERY. doi:10.1007/s13399-024-05338-2.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., De Vargas, C., Decelle, J., Del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., and Christen, R. 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic acids research **41**(Database issue). doi:10.1093/NAR/GKS1160.

He, X., Wang, Y.Y., Tai, M.H., Lin, A., Owyong, S., Li, X., Leong, K., Yusof, M.L.M., Ghosh, S., and Wang, C.H. 2022. Integrated applications of water hyacinth biochar: A circular economy case study. JOURNAL OF CLEANER PRODUCTION **378**. doi:10.1016/j.jclepro.2022.134621.

Hernández-Fernández, L., Vázquez, J.G., Hernández, L., Campbell, R., Martínez, J., Hajari, E., Zayas, R.G.D., Zevallos-Bravo, B.E., Acosta, Y., and Lorenzo, J.C. 2024. Use of Euclidean distance to evaluate Pistia stratiotes and Eichhornia crassipes as organic fertilizer amendments in Capsicum annuum. ACTA PHYSIOLOGIAE PLANTARUM **46**(2). doi:10.1007/s11738-024-03652-w.

Huang, H., Reddy, N.G., Huang, X.L., Chen, P.N., Wang, P.Y., Zhang, Y.T., Huang, Y.X., Lin, P., and Garg, A. 2021. Effects of pyrolysis temperature, feedstock type and compaction on water retention of biochar amended soil. SCIENTIFIC REPORTS **11**(1). doi:10.1038/s41598-021-86701-5.

Jutakanoke, R., Intaravicha, N., Charoensuksai, P., Mhuantong, W., Boonnorat, J., Sichaem, J., Phongsopitanun, W., Chakritbudsabong, W., and Rungarunlert, S. 2023. Alleviation of soil acidification and modification of soil bacterial community by biochar derived from water hyacinth Eichhornia crassipes. SCIENTIFIC REPORTS **13**(1). doi:10.1038/s41598-023-27557-9.

Khan, A., Mfarrej, M.F.B., Tariq, M., Asif, M., Nadeem, H., Siddiqui, M.A., Hashem, M., Alamri, S., and Ahmad, F. 2022. Supplementing Pochonia chlamydosporia with botanicals for management of Meloidogyne incognita infesting chickpea. ACTA AGRICULTURAE SCANDINAVICA SECTION B-SOIL AND PLANT SCIENCE **72**(1): 164-175. doi:10.1080/09064710.2021.2003853.

Khatun, M., Hossain, M., and Joardar, J.C. 2024. Quantifying the acceptance and adoption dynamics of biochar and co-biochar as a sustainable soil amendment. PLANT SCIENCE TODAY **11**(2): 307-317. doi:10.14719/pst.3242.

Legendre, P., Oksanen, J., and ter Braak, C.J.F. 2011. Testing the significance of canonical axes in redundancy analysis. Methods in Ecology and Evolution **2**(3): 269-277. doi:https://doi.org/10.1111/j.2041-210X.2010.00078.x.

Liu, C., Cui, Y., Li, X., and Yao, M. 2021. microeco: an R package for data mining in microbial community ecology. FEMS Microbiology Ecology **97**(2). doi:10.1093/femsec/fiaa255.

Louca, S., Parfrey, L.W., and Doebeli, M. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science **353**(6305): 1272-1277. doi:10.1126/SCIENCE.AAF4507/SUPPL_FILE/LOUCA.SM.PDF.

Love, M.I., Huber, W., and Anders, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology **15**(12): 550. doi:10.1186/s13059-014-0550-8.

Lustenhouwer, N., Maynard, D.S., Bradford, M.A., Lindner, D.L., Oberle, B., Zanne, A.E., and Crowther, T.W. 2020. A trait-based understanding of wood decomposition by fungi. Proceedings of the National Academy of Sciences **117**(21): 11551-11558. doi:doi:10.1073/pnas.1909166117.

Majee, S., Halder, G., and Mandal, T. 2019. Formulating nitrogen-phosphorous-potassium enriched organic manure from solid waste: A novel approach of waste valorization. PROCESS SAFETY AND ENVIRONMENTAL PROTECTION **132**: 160-168. doi:10.1016/j.psep.2019.10.013.

Mei, G.X., Kumar, H., Huang, H., Cai, W.L., Reddy, N.G., Chen, P.N., Garg, A., and Ganeshan, S.P. 2021. Desiccation Cracks Mitigation Using Biomass Derived Carbon Produced from Aquatic Species in South China Sea. WASTE AND BIOMASS VALORIZATION **12**(3): 1493-1505. doi:10.1007/s12649-020-01057-7.

Oberacker, P., Stepper, P., Bond, D.M., Höhn, S., Focken, J., Meyer, V., Schelle, L., Sugrue, V.J., Jeunen, G.J., Moser, T., Hore, S.R., von Meyenn, F., Hipp, K., Hore, T.A., and Jurkowski, T.P. 2019. Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid extraction and manipulation. PLOS Biology **17**(1): e3000107-e3000107. doi:10.1371/JOURNAL.PBIO.3000107.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J., and Suggests, M. 2007. The vegan package. Community ecology package **10**: 631-637.

Paterson, E., and Sim, A. 2013. Soil-specific response functions of organic matter mineralization to the availability of labile carbon. Global Change Biology **19**(5): 1562-1571. doi:https://doi.org/10.1111/gcb.12140.

Patra, R.K., Behera, D., Mohapatra, K.K., Sethi, D., Mandal, M., Patra, A.K., and Ravindran, B. 2022. Juxtaposing the quality of compost and vermicompost produced from organic wastes

amended with cow dung. ENVIRONMENTAL RESEARCH **214**. doi:10.1016/j.envres.2022.114119.

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B.D., Clemmensen, K.E., Kauserud, H., Nguyen, N., Kjøller, R., Bates, S.T., Baldrian, P., Frøslev, T.G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.O., Järv, H., Madrid, H., Nordén, J., Liu, J.K., Pawlowska, J., Põldmaa, K., Pärtel, K., Runnel, K., Hansen, K., Larsson, K.H., Hyde, K.D., Sandoval-Denis, M., Smith, M.E., Toome-Heller, M., Wijayawardene, N.N., Menolli, N., Reynolds, N.K., Drenkhan, R., Maharachchikumbura, S.S.N., Gibertoni, T.B., Læssøe, T., Davis, W., Tokarev, Y., Corrales, A., Soares, A.M., Agan, A., Machado, A.R., Argüelles-Moyao, A., Detheridge, A., de Meiras-Ottoni, A., Verbeken, A., Dutta, A.K., Cui, B.K., Pradeep, C.K., Marín, C., Stanton, D., Gohar, D., Wanasinghe, D.N., Otsing, E., Aslani, F., Griffith, G.W., Lumbsch, T.H., Grossart, H.P., Masigol, H., Timling, I., Hiiesalu, I., Oja, J., Kupagme, J.Y., Geml, J., Alvarez-Manjarrez, J., Ilves, K., Loit, K., Adamson, K., Nara, K., Küngas, K., Rojas-Jimenez, K., Bitenieks, K., Irinyi, L., Nagy, L.L., Soonvald, L., Zhou, L.W., Wagner, L., Aime, M.C., Öpik, M., Mujica, M.I., Metsoja, M., Ryberg, M., Vasar, M., Murata, M., Nelsen, M.P., Cleary, M., Samarakoon, M.C., Doilom, M., Bahram, M., Hagh-Doust, N., Dulya, O., Johnston, P., Kohout, P., Chen, Q., Tian, Q., Nandi, R., Amiri, R., Perera, R.H., dos Santos Chikowski, R., Mendes-Alvarenga, R.L., Garibay-Orijel, R., Gielen, R., Phookamsak, R., Jayawardena, R.S., Rahimlou, S., Karunarathna, S.C., Tibpromma, S., Brown, S.P., Sepp, S.K., Mundra, S., Luo, Z.H., Bose, T., Vahter, T., Netherway, T., Yang, T., May, T., Varga, T., Li, W., Coimbra, V.R.M., de Oliveira, V.R.T., de Lima, V.X., Mikryukov, V.S., Lu, Y., Matsuda, Y., Miyamoto, Y., Kõljalg, U., and Tedersoo, L. 2020. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Diversity **105**(1): 1-16. doi:10.1007/S13225-020-00466-2/FIGURES/3.

Porazinska, D.L., Giblin-Davis, R.M., Faller, L., Farmerie, W., Kanzaki, N., Morris, K., Powers, T.O., Tucker, A.E., Sung, W., and Thomas, W.K. 2009. Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. Molecular Ecology Resources **9**(6): 1439-1450. doi:10.1111/J.1755-0998.2009.02611.X.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research **41**(Database issue): D590-D590. doi:10.1093/NAR/GKS1219.

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramdas, M.G., Manjunath, B.L., Pratap, S.N., Ramesh, R., Verma, R.R., Marutrao, L.A., Ruenna, D., Natasha, B., and Rahul, K. 2017. Effect of organic and inorganic sources of nutrients on soil microbial activity and soil organic carbon build-up under rice in west coast of India. ARCHIVES OF AGRONOMY AND SOIL SCIENCE **63**(3): 414-426. doi:10.1080/03650340.2016.1213813.

Renaud, G., Stenzel, U., Maricic, T., Wiebe, V., and Kelso, J. 2015. deML: robust demultiplexing of Illumina sequences using a likelihood-based approach. Bioinformatics **31**(5): 770-772. doi:10.1093/BIOINFORMATICS/BTU719.

Rop, K., Mbui, D., Njomo, N., Karuku, G.N., Michira, I., and Ajayi, R.F. 2019. Biodegradable water hyacinth cellulose-graft-poly(ammonium acrylate-co-acrylic acid) polymer hydrogel for potential agricultural application. HELIYON **5**(3). doi:10.1016/j.heliyon.2019.e01416.

Sansupa, C., Wahdan, S.F.M., Hossen, S., Disayathanoowat, T., Wubet, T., and Purahong, W. 2021. Can We Use Functional Annotation of Prokaryotic Taxa (FAPROTAX) to Assign the Ecological Functions of Soil Bacteria? Applied Sciences **11**(2): 688. Available from https://www.mdpi.com/2076-3417/11/2/688 [accessed.

Sikder, M.M., Vestergård, M., Sapkota, R., Kyndt, T., and Nicolaisen, M. 2020. Evaluation of Metabarcoding Primers for Analysis of Soil Nematode Communities. Diversity 2020, Vol. 12, Page 388 **12**(10): 388-388. doi:10.3390/D12100388.

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., and Nishijima, M. 2014. Development of a Prokaryotic Universal Primer for Simultaneous Analysis of Bacteria and Archaea Using Next-Generation Sequencing. PLOS ONE **9**(8): e105592-e105592. doi:10.1371/JOURNAL.PONE.0105592.

van Bruggen, A.H.C., Goss, E.M., Havelaar, A., van Diepeningen, A.D., Finckh, M.R., and Morris, J.G. 2019. One Health - Cycling of diverse microbial communities as a connecting force for soil, plant, animal, human and ecosystem health. Science of The Total Environment **664**: 927-937. doi:https://doi.org/10.1016/j.scitotenv.2019.02.091.

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., and Pérez-Clemente, R.M. 2020. Root exudates: from plant to rhizosphere and beyond. Plant Cell Reports **39**(1): 3-17. doi:10.1007/s00299-019-02447-5.

Weedon, J.T., Cornwell, W.K., Cornelissen, J.H.C., Zanne, A.E., Wirth, C., and Coomes, D.A. 2009. Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? Ecology Letters **12**(1): 45-56. doi:https://doi.org/10.1111/j.1461-0248.2008.01259.x.

Wu, S.-H., Huang, B.-H., Huang, C.-L., Li, G., and Liao, P.-C. 2018. The Aboveground Vegetation Type and Underground Soil Property Mediate the Divergence of Soil Microbiomes and the Biological Interactions. Microbial Ecology **75**(2): 434-446. doi:10.1007/s00248-017-1050-7.

Yan, Z., Xiong, C., Liu, H., Singh, B.K., and Brajesh Singh, C.K. 2022. Sustainable agricultural practices contribute significantly to One Health. Journal of Sustainable Agriculture and Environment **1**(3): 165-176. doi:10.1002/SAE2.12019.