

Research paper

Value of food waste-derived fertilisers on soil chemistry, microbial function and crop productivity

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ABSTRACT

Food waste management through composting and anaerobic digestion are sustainable waste management processes that can promote sustainable agricultural practices by recycling nutrients, reducing reliance on synthetic fertilisers, and conditioning soils. However, compost and digestate (anaerobic digestate effluent) are low nutrient value fertilisers, limiting their use within agriculture due to the large volumes required for land application. Alternatively, food waste-derived fertilisers could be used alongside chemical fertilisers to improve crop growth further and reduce demand on synthetic fertilisers. Therefore, this study evaluated the impact of food waste-derived fertilisers (compost, liquid digestate (LD), and solid digestate (SD)) on plant growth (Ryegrass) when applied at a rate of 50 kg N-ha⁻¹ in combination with synthetic fertiliser (Urea Ammonium Nitrate (UAN)) at a rate of 0 or 50 kg N-ha⁻¹. Ryegrass growth, soil chemistry, and rhizosphere microbial communities were investigated. SD in combination with UAN yielded the largest shoot biomass, while sole UAN at any application rate was not significantly different from the control. Beneficial plant growth phyla were in higher abundance in all treatments amended with food waste-derived fertilisers, and putative genes encoded for N fixing were found in higher abundances in all soil amendments compared to control. This highlights the value of food waste-derived fertilisers within agriculture. However, while drying would logically reduce costs associated with digestate transport, ammonia volatilisation led to significant N losses, reducing the fertiliser value of SD. Future studies should explore methods to mitigate ammonia valorisation to enhance the final fertiliser value of SD.

1. Introduction

Currently, the conventional disposal of food waste (FW) to landfills and its incineration contribute ~20 % of global greenhouse gas emissions, while contributing to groundwater and surface water contamination (FAO, 2011; O'Connor et al., 2021). Moreover, the current disposal of food waste leads to a major loss of nutrients including nitrogen that can otherwise be re-used in agriculture. Therefore, sustainable food waste valorisation methods, such as composting or anaerobic digestion for nutrient recycling, are essential to close the sustainable circular bioeconomy loop. A circular bioeconomy can be defined as the conversion of value-added products from renewable waste streams by various industries (e.g., food waste into bioplastics or methane)

(Stegmann et al., 2020). Globally, nitrogen (N) fertiliser use has increased from 105 million tonnes in 2016 to 112 million tonnes in 2022 due to the increasing need for food production to meet the demand for growing population (FAO, 2022). As synthetic fertilisers are derived from fossil-fuel resources, the increase in fertiliser production, particularly N fertilisers, contributes to an increasing carbon (C) footprint in agriculture (FAO, 2011). The production of N fertilisers, such as urea ammonium nitrate (UAN), which is used extensively in arable farming in Australia is estimated to consume approximately 2 % of the world's energy (Walling and Vaneekhaute, 2020). Therefore, while recycling food waste nutrients is beneficial for agriculture, food waste-derived fertilisers could reduce the C footprint of agricultural systems by reducing demand for synthetic fertilisers.

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Composting and anaerobic digestion of food waste is the most common valorisation practices for food waste management; however, anaerobic digestion is a higher-value process as it extracts methane from the food waste that can be used as a biofuel (O'Connor et al., 2021). Moreover, anaerobic digestate has been shown to reduce greenhouse gas emissions more than composting due to the production and capture of biogas (Lin et al., 2018). However, raw liquid digestate is often considered a cost liability for many anaerobic digestion facilities, despite its imbedded C and nutrient value. This is partly due to its bulky nature, with a high moisture content, diluting its nutrient value, and increasing its associated costs of storage, transport, and land application (Lin et al., 2018). Therefore, this study aims at examining the fertiliser value of using dried solid digestate as a comparison to raw liquid digestate.

Digestate has shown its benefit in agriculture due to higher available N values than compost. However, limited studies compare the agricultural utilisation of compost and digestate (Lin et al., 2018). Therefore, this study aims to bridge this knowledge gap. Composts and digestates derived from food waste have an array of macro- and micro-nutrients, plant-promoting compounds, and microbes (Ren et al., 2020). While synthetic N fertilisers elevate available N concentrations, which promote plant growth, the organic matter in compost and anaerobic digestate can reduce the leaching (NO_3^- , NH_4^+ and PO_4^-) and gaseous emissions (N_2O and NH_3) likely to result from synthetic fertiliser application (Alessandrino et al., 2021). Furthermore, this organic matter increases soil microbial biomass, immobilising excessive N that may result when applying synthetic fertiliser (Holub et al., 2020). For digestate, this microbial N is then released through ammonification and organic N is converted back into NH_4^+ , slowing the rate of N release into the soil (Lin et al., 2018). Additionally, the humic acids and organic matter found in the food waste fertilisers including digestate and composts can provide co-benefits including improvements in soil structure, moisture retention, and nutrient cycling (Fernández-Bayo et al., 2017).

However, while these food waste-derived fertilisers promote soil health, can also cause strong nutrient imbalances and increased soil alkalinity and salinity under certain conditions (Zikeli et al., 2017). Furthermore, while limited studies have shown the positive combined effects of organic amendments and fertiliser on plant growth (de França et al., 2021), studies on food waste-derived fertilisers and their interaction with N fertiliser remain limited. The addition of organic amendment and inorganic N to soils can also influence nitrification and denitrification processes. Studies have indicated that organic amendments with low C/N ratio and inorganic N applied to acidic soils can cause a shift from nitrification to denitrification (N_2O and N_2 emission) (Wang et al., 2023). However, there are suggestions that the combination of both organic and inorganic fertiliser can reduce denitrification by decreasing abundances of nirK putative genes (Yang et al., 2022).

This study aims to compare conventional food waste fertilisers on soil properties, plant growth and microbial community structure and explore their potential as a N fertiliser enhancer, reducing the demand of synthetic N fertilisers in agriculture. The specific objectives were: (i) to examine and compare the effect of three food waste fertilisers (compost, solid digestate and liquid digestate) on soil properties; (ii) to explore the interactions between food waste fertiliser and synthetic N fertiliser as measured by plant growth and plant characteristics; and (iii) to examine the relationship between functional genes, C & N mineralisation rates, with the combination of food waste fertilisers and synthetic N fertiliser. The research outcomes from this study will help to increase food waste recycling to higher-value nutrient fertilisers. It is hypothesised that organic amendments will enhance soil characteristics and nutrients, increasing plant growth; the combination of organic amendments and inorganic N will have synergistic effects, increasing plant growth further. It is also hypothesised that organic amendment addition will enhance C and N cycling, and introduce beneficial functional genes for carbon and nitrogen cycles, along with a high r-strategist bacterial community to support plant growth.

2. Materials and methods

2.1. Collection of food waste fertilisers and soil

Food waste-derived compost and raw liquid digestate (see Bühlmann et al. (2022)) were sourced from a mixed-food waste anaerobic digestion and compost facility located at Jandakot, Western Australia. SD was derived by screened LD at <2 mm then evaporated at 60 °C. Liquid urea ammonium nitrate (UAN), 32 % N was used as a positive control. Soil was collected from a pasture paddock that was located at 32°28'44.1"S 116°59'56.2"E, near Pingelly, Western Australia. The soil experiences a Mediterranean climate with hot summers, and dry and cool winters. The Australian soil characterisation of the soil was a Eutrophic Kurosol (Isbell, 1996). CSBP® Soil and Plant Analysis Laboratory characterised the soil and food waste fertilisers used in this study (Table 1).

2.2. Experimental design

The plant growth pot experiment consisted of a temperature-controlled glasshouse (20–26 °C) with four replicates ($n = 4$) for each treatment. The experimental design consisted of factorial randomised block design with nine treatments (Table S1; Supplementary material). The treatments include control, urea ammonium nitrate applied at 50 kg ha⁻¹ (UAN 50), urea ammonium nitrate applied at 100 kg ha⁻¹ (UAN 100), liquid digestate applied at 50 kg ha⁻¹ (LD), liquid digestate applied at 50 kg ha⁻¹ + UAN applied at 50 kg ha⁻¹ (LD + UAN 50), solid digestate applied at 50 kg ha⁻¹ (SD), solid digestate applied at 50 kg ha⁻¹ + UAN applied at 50 kg ha⁻¹ (SD + UAN 50) (All treatments are displayed in Table S1; Supplementary material). Treatments were applied at rates of 0, 50, and 100 kg·ha⁻¹ total N. Organic soil amendments were applied at the rate of 50 kg·ha⁻¹ total N. UAN was also applied at the rate of 50 kg·ha⁻¹ total N. A combination of organic amendment + UAN was applied at 100 kg·ha⁻¹ total N. A positive control (UAN 100) was added at 100 kg ha⁻¹ total N to mimic the rate of organic amendments + UAN. Pots were lined with a polyethylene bag and contained 800 g soil. The moisture content of the pots was maintained at 75 % field capacity during germination and increased to 100 % during plant growth period. Eight seeds of ryegrass (*Lolium perenne* c.v. *Banquet*) were germinated, and after 14 days, they were thinned to 4 plants per pot. Plants were grown for 56 days during the period of 18/07/2022 to 12/09/2022.

Table 1

Characteristics of soil, urea ammonium nitrate (UAN), and food waste fertiliser products. Values represent mean.

Parameter	Soil	UAN	Compost	Liquid digestate	Solid digestate
Total C (%)	7.04	0	15.28	0.98	36.93
Total N (%)	0.36	32	1.33	0.39	5.13
C/N	19.6	–	11.5	2.5	7.2
Total P (%)	–	0	0.21	0.04	1.70
Total K (%)	0.02	0	0.47	0.14	5.06
Ammonium-N (mg/kg)	2	77,000	8	2300	157
Nitrate-N (mg/kg)	5	77,000	580	33	3
Available-P (mg/kg)	45	0	24	0.01	741
Na (%)	–	0	0.20	0.17	3.76
S (mg/kg)	20.3	0	0.18	0.01	0.75
EC (mS/cm)	0.098	–	2.98	27.52	15.74
pH (CaCl ₂)	4.8	–	7.30	7.92	8.28
pH (H ₂ O)	5.9	6.5–7.5	7.51	7.97	9.00
SD total N recovery					
SD from LD evaporation (%)	–	–	–	–	32.5
SD NH ₄ ⁺ recovery from LD evaporation (%)	–	–	–	–	0.18

2.3. Analysis of plant and soil properties

At experimental termination, pots were harvested, and plant shoots were dried at 70 °C for 72 h and weighed for dry shoot biomass. The shoot nutrients and root morphology characteristics of root length, root surface area, average root diameter and root volume were measured as described by Mickan et al. (2022). The interactions between organic fertiliser and inorganic fertiliser (UAN) were derived from shoot biomass according to Rietra et al. (2017):

$$\left[\frac{y_{ab}}{y_0} \right] > \left[\frac{y_a}{y_0} \right] \times \left[\frac{y_b}{y_0} \right] \quad (\text{Positive interaction--synergism}) \quad (1)$$

$$\left[\frac{y_{ab}}{y_0} \right] < \left[\frac{y_a}{y_0} \right] \times \left[\frac{y_b}{y_0} \right] \quad (\text{Negative interaction--antagonism}) \quad (2)$$

where y_{ab} is the shoot biomass of organic fertiliser + UAN, y_a is the shoot biomass of organic fertiliser, y_b is the shoot biomass of UAN, and y_0 is the shoot biomass of control.

Soil properties measured within the experiment include pH (H₂O and CaCl₂), electrical conductivity (EC), Ammonium (NH₄⁺-N), nitrate (NO₃⁻-N), and microbial biomass carbon (MBC). NH₄⁺ and NO₃⁻ were determined colourimetrically, and MBC was determined using fumigation. These soil properties were determined using the same method and analytical equipment as Srivastava et al. (2023).

2.4. DNA extraction, amplification, and bioinformatics analysis

Following the manufacturers instruction, 0.25 g of soil sample from each treatment was used to extract DNA using the Mo Bio Powersoil DNA isolation kit (Qiagen., Hilden, NRW). DNA was quantified per sample using a Qubit (Thermo Fisher Scientific Inc., Waltham, MA). After extraction, samples were stored at -20 °C prior to further analysis. Bacterial 16S rRNA gene fragments were amplified from DNA samples using universal core bacterial primers 27 F and 519R (Mori et al., 2014) modified with Golay barcodes (Caporaso et al., 2012). DNA sequencing and bioinformatics was performed on the Illumina MiSeq platform with 2 × 300 bp Paired-End V3 at the Australian Genome Research Facility, following the same bioinformatic pipeline as Ren et al. (2020). Using PEAR (version 0.9.5) (Zhang et al., 2014), the assembly of paired-end reads was achieved by aligning the forward and reverse sequences. Primers were identified and subsequently trimmed. Quantitative Insights into Microbial Ecology (QIIME 1.8) software (Caporaso et al., 2010), Usearch (version 8.0.1623) and UPRASE tools (Edgar et al., 2011) were used to process trimmed sequences. Full-length duplicate sequences were removed and sorted according to abundance, and sequences were quality filtered using Usearch tools. Unique reads were discarded, and sequences were clustered according to a chimera, and filtered using a reference database (rdp_gold). Reads were aligned to operational taxonomic units (OTUs) with at least 97 % similarity to determine the read count for each OTU. Post-processing, all samples contained a minimum of 30,000 sequences. The taxonomy was classified using QIIME, utilizing the Greengenes database (Version 13.8, Aug 2013) (DeSantis et al., 2006).

The study utilised Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (Langille et al., 2013) to analyse potential microbial genes that encode enzymes for carbon and nitrogen cycling. 16S rRNA data from bacterial communities was used to predict metagenomic content. In addition, PICRUSt2 (Douglas et al., 2020), along with other tools such as EPA-ng (Barbera et al., 2019), Gappa (Czech et al., 2020), castor (Louca and Doebeli, 2018), and MinPath (Ye and Doak, 2009), was employed to map the filtered sequence reads to functional ortholog groups (KEGG orthologs; KOs) and to deduce functional pathways using MetaCyc database (Caspi et al., 2016). The metagenome predictions was tested using the Nearest Sequenced Taxon Index. The precision of the results is tied to the

proximity of the sample to a closely related representative bacterial genome, with lower values indicating a more exact match (Langille et al., 2013).

2.5. Statistical analysis

All statistical testing was carried out in R, version 3.4.1 (Ihaka and Gentleman, 1996). One-way analysis of variance (ANOVA) was conducted for plant growth, soil, and microbiological variables. If ANOVA was significant, a post-hoc Tukey's HSD ($p < 0.05$) was performed. The effect of application rate and the type of fertiliser on plant growth and soil parameters was quantified using a principal component analysis (PCA). Beta diversity of the bacteria community was assessed with permutational multivariate analysis of variance (PERMANOVA) using distance matrices (Adonis function). Canonical correspondence analysis (CCA) was used to show the relationship between soil bacteria phyla and genera on plant and soil variables. Pearson's correlation of soil, plant variables, and C and N functional gene abundances was used to demonstrate the relationship between the treatment and measured variables.

3. Results

3.1. Plant growth

3.1.1. Shoot and root biomass

Fig. 1a and b outline the influence of the compost, LD, and SD applied singly or in combination with UAN on the dry shoot and root biomass. LD and SD had significantly higher dry shoot biomass than control, UAN 50, UAN 100 and compost. SD + UAN 50 had significantly higher shoot biomass than all treatments and was 310 % higher than the control. LD and SD applied singly had similar shoot biomass whereas compost had shoot biomass that was not significantly different to the control. The combination of UAN with compost or SD led to a significant increase in shoot biomass. In contrast, there was no significant difference between the co-application of UAN and LD or LD applied alone. Based on shoot biomass, positive interaction (synergism, Eq. (1)) was observed for compost + UAN 50 (3.21 > 1.59) and SD + UAN 50 (4.10 > 3.07), respectively (Table 2). However, a negative interaction (antagonism) was observed with LD + UAN 50 (2.15 < 2.97).

For root biomass, only SD had significantly higher root biomass than control. Compost, SD, and SD + UAN 50 had significantly higher root biomass than UAN at either application rate. Control had significantly higher root to shoot ratio than when applied with UAN (SI-Fig. 1). Moreover, the application of UAN to compost and SD saw a significant increase in root to shoot ratio. The application of UAN to LD did not show a significant difference.

3.1.2. Root characteristics

Fig. 1c to f shows how all root characteristics had similar pattern to dry root biomass response to various treatments; however, with different significant differences shown by Tukey's HSD. Compost had significantly higher root length, root surface area, average root diameter, and root volume than control and UAN. SD, and SD + UAN 50 had significantly higher root length, root surface area, average root diameter, and root volume than control, UAN, and LD + UAN 50. There was no significant difference amongst LD + UAN 50, control, UAN 50, UAN 100, and LD + UAN 50. Applying UAN to organic amendments decreased the root length, root surface area, average root diameter, and root volume. However, there is no significant difference between organic amendments and organic amendments + UAN.

3.1.3. Plant nutrients

Table 3 shows the shoot nutrients of each treatment. As expected, shoot N content increased with UAN application as well as the application of LD compared to control. There was no significant difference in

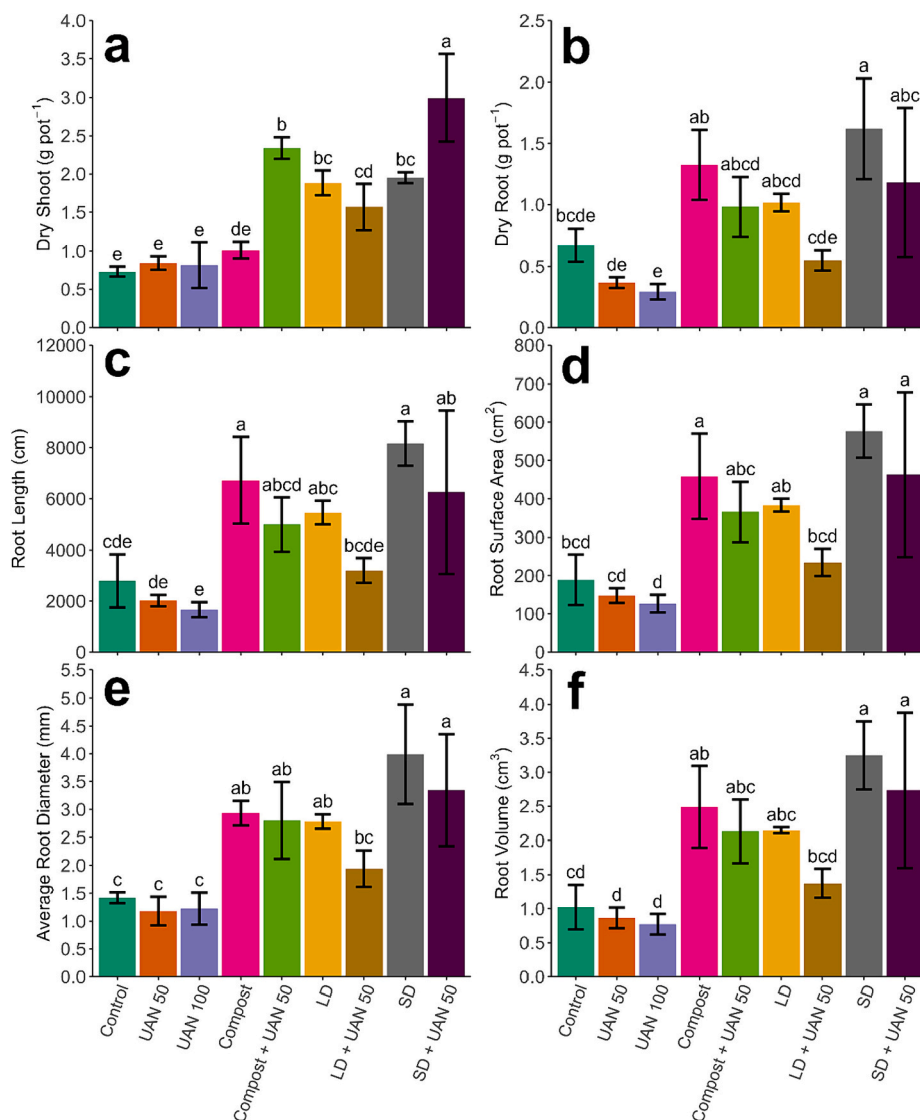


Fig. 1. Plant characteristics at harvest of ryegrass of nine different treatments. LD = Liquid Digestate; SD = Solid Digestate; UAN 50 = Urea Ammonium Nitrate applied at a rate of 50 kg total N ha⁻¹; UAN 100 = Urea Ammonium Nitrate applied at a rate of 100 kg total N ha⁻¹. Bars represent mean and error bars represent standard deviation (n = 4). Tukey's HSD represents significant differences between treatments.

Table 2

Dry shoot biomass synergism/antagonism parameters of organic amendments + UAN. y_{ab} is the shoot biomass of organic fertiliser + UAN, y_a is the shoot biomass of organic fertiliser, y_b is the shoot biomass of UAN, and y_0 is the shoot biomass of control.

Treatment	$\frac{y_{ab}}{y_0}$	$\frac{y_a}{y_0}$	$\frac{y_b}{y_0}$	$\frac{y_a}{y_0} \times \frac{y_b}{y_0}$	Synergism/antagonism
Compost + UAN 50	3.21	1.38	1.15	1.59	Synergism $\left[\frac{y_{ab}}{y_0} > \frac{y_a}{y_0} \times \frac{y_b}{y_0}\right]$
LD + UAN 50	2.15	2.58	1.15	2.97	Antagonism $\left[\frac{y_{ab}}{y_0} < \frac{y_a}{y_0} \times \frac{y_b}{y_0}\right]$
SD + UAN 50	4.10	2.67	1.15	3.07	Synergism $\left[\frac{y_{ab}}{y_0} > \frac{y_a}{y_0} \times \frac{y_b}{y_0}\right]$

shoot N when compost or SD was applied compared to control. For shoot P, the application of amendments was not significantly different to the control. There was a decrease in shoot P from 0.28 % to 0.16 % when UAN was added to compost. Shoot K varied significantly between treatments, ranging from 3.29 % (compost) to 4.85 % (UAN 50). For the shoot micronutrients of Na, Ca, Mg, S, Mn, and Zn, the values varied slightly from the control amongst all treatments.

3.2. Analysis of amendments and soil properties

Table 1 shows that SD had the highest N, P, K and S concentrations relative to the same total N application. SD and LD were alkaline in nature (pH 9 and 7.97, respectively). After LD dewatering, only 0.18 % NH₄⁺ from LD was retained. Therefore, NH₄⁺ concentration was much higher in LD (2300 mg/kg) compared to SD (157 mg/kg). 99.8 % (w/w basis) of NH₄⁺ was lost from LD when it was evaporated. NH₄⁺ concentrations were also low in compost (8 mg/kg).

Fig. 2e and f shows the pH and EC in the soil post-harvest. The application of UAN to treatments resulted in a significant decrease in soil pH (except for compost), indicating acidification of soil. SD and compost showed reduced and no acidification, respectively when UAN was

Table 3

Nutrient content of plant shoot biomass. UAN 50 = Urea Ammonium Nitrate applied at a rate of 50 kg total N/ha; UAN 100 = Urea Ammonium Nitrate applied at a rate of 100 kg total N/ha; LD = Liquid Digestate; SD = Solid Digestate. Values represent mean with standard deviation. ANOVA represent F-value with *p*-value indicated as *. Significance codes are 0 *****, 0.001 ***, 0.01 **, 0.05 ', 0.1 ' ', 1.

Treatment	Nutrient								
	N (%)	P (%)	K (%)	Na (%)	Ca (%)	Mg (%)	S (%)	Mn (mg/kg)	Zn (mg/kg)
Control	1.93c ±0.24	0.21abc ±0.01	3.53c ±0.17	0.37c ±0.06	0.40c ±0.03	0.29bc ±0.03	0.19c ±0.02	171a ±21.45	17.8c ±3.36
UAN 50	5.61a ±0.10	0.17bc ±0.03	4.85a ±0.18	0.43abc ±0.05	0.58ab ±0.07	0.40a ±0.04	0.29ab ±0.02	133ab ±26.20	19.5bc ±3.17
UAN 100	5.97a ±0.16	0.18bc ±0.01	4.44ab ±0.34	0.37c ±0.09	0.66a ±0.14	0.38ab ±0.07	0.31a ±0.01	114ab ±19.40	23.0bc ±2.84
Compost	1.72c ±0.21	0.28a ±0.04	3.29c ±0.37	0.38c ±0.06	0.54abc ±0.06	0.31bc ±0.02	0.16c ±0.02	124ab ±22.27	18.3bc ±2.07
Compost + UAN 50	4.36b ±0.21	0.16c ±0.03	3.39c ±0.35	0.67a ±0.07	0.45bc ±0.04	0.31abc ±0.02	0.25b ±0.02	97b ±19.27	20.0bc ±3.98
LD	4.16b ±0.41	0.18bc ±0.02	4.08abc ±0.14	0.65ab ±0.19	0.42c ±0.02	0.35ab ±0.02	0.28ab ±0.01	132ab ±7.63	19.9bc ±0.77
LD + UAN 50	5.90a ±0.28	0.21abc ±0.02	4.79a ±0.40	0.42bc ±0.07	0.48bc ±0.02	0.32abc ±0.01	0.29a ±0.01	108b ±4.91	26.3ab ±2.25
SD	2.10c ±0.10	0.25ab ±0.06	3.58bc ±0.32	0.46abc ±0.02	0.41c ±0.04	0.25c ±0.03	0.15c ±0.02	123ab ±40.70	16.0c ±4.75
SD + UAN 50	4.45b ±0.70	0.19bc ±0.01	4.82a ±0.42	0.62abc ±0.10	0.45bc ±0.04	0.32abc ±0.02	0.27ab ±0.02	131ab ±12.45	31.8a ±0.39
ANOVA	85.96 ***	5.46***	12.86 ***	5.68 ***	6.68 ***	5.39 ***	38.49 ***	2.72*	8.38 ***

applied compared to the other treatments. Both compost and SD significantly increased soil pH compared to control. There was no significant change in pH when LD was applied compared to control. However, LD showed the greatest change in pH amongst organic amendments when UAN was applied. The EC only showed a significant increase in UAN 100 and LD + UAN 50, relative to control.

Fig. 2a and b depict NH_4^+ and NO_3^- in the soil post-harvest. NH_4^+ and NO_3^- concentration was positively correlated (SI-Fig. 14). NH_4^+ concentrations were lower than NO_3^- across treatments. UAN 100 and LD + UAN 50 had significantly higher NH_4^+ levels compared to other treatments within the soil; others were close to zero. Similar results were shown for NO_3^- samples, with elevated levels in UAN 50, UAN 100, and LD + UAN 50. Control and organic amendments showed depleted NH_4^+ and NO_3^- compared to a selected few UAN amended soils. Fig. 2c also shows the DOC levels in soil of all treatments. SD + UAN 50 had the highest levels of DOC compared to all other treatments. Compost and compost + UAN 50 had the lowest levels of DOC.

3.3. Microbiology analyses

Fig. 2d shows the MBC levels in the soil of all treatments. Control had significantly higher levels of MBC compared to all other treatments. Compost + UAN 50 had lower levels of MBC than most treatments but was not significantly different from LD and LD + UAN 50. Fig. 3 shows the alpha diversity indices of the treatments. There was significantly higher fisher's alpha diversity in compost + UAN 50, LD, LD + UAN 50 and SD compared to the control (Fig. 3a). There was no significant difference of UAN treatments with control and addition of organic amendments, except for compost. Similar results were shown in OTU richness (Fig. 3b). Control and compost had the highest species evenness amongst the treatments and were higher than the other organic amendments and organic amendments + UAN 50. SD had the lowest evenness however, had large standard error (Fig. 3c). Similarly, this can be seen in the inverse Simpson index (Fig. 3d). There were no significant differences between treatments except for SD in the inverse Simpson index.

The application of organic amendment and UAN resulted in a decrease of MBC overall. The application of soil amendments significantly influenced the soil bacteria community (Fig. 4). Actinobacteria, Chloroflexi, and Proteobacteria were the most dominant soil bacteria present, accounting for ~70–85 % of total OTUs. There was an increase

in Gemmatimonadetes, Proteobacteria, and Patescibacteria and a decrease in Firmicutes and Actinobacteria when amendments were applied to soil. UAN treatments resulted in a decrease in Acidobacteria. A CCA on bacterial phyla community and soil and plant variables (Fig. 5a) showed increased pH and dry shoot were associated with all organic amendments, and Proteobacteria, Patescibacteria, Bacteroidetes, Gemmatimonadetes, and Verrucomicrobia were most associated with these treatments. UAN treatments were associated with an increase in mineral N and the abundance of Chloroflexi. Control was associated with the highest level of MBC and was dominated by Firmicutes and Actinobacteria compared to other treatments. The CCA on bacterial genera community and plant variables (Fig. 5b) were similar to Fig. 5a, with increased pH and dry shoot were associated with all organic amendments. All uncultured Ktedonobacteraceae, Mycobacterium, Gemmatimonas and unspecified were associated with these treatments. Control was dominated by Conexibacter and Streptomyces. The relative abundances of the soil genera can be observed in SI-Fig. 6.

3.3.1. In-silico gene inference

The relative abundances of putative C and N cycling genes were evaluated (Fig. 6). For C cycling, putative genes encoded to degrade hemicellulose (beta-galactosidase; *LacZ*), chitin (chitinase; *ChiC*) and starch (glucoamylase; *SusB*) were found to higher abundance in organic, UAN and organic + UAN treatments than control. LD and SD treatments had higher levels of lignin degrading gene abundance (catalase; *SrPA*) and decreased abundance of the gene encoded for cellulose degradation (endoglucanase; *bcsZ*) compared to the other treatments. For N cycling, putative genes involved in N fixation (nitrogenase; *nifD*), nitrification (hydroxylamine oxidoreductase; *HaO*) and denitrification (nitrous-oxide reductase; *nosZ*), were found to higher abundance in organic, UAN and organic + UAN treatments than control. Notably, SD and SD + UAN 50, had elevated abundances of denitrification putative genes of nitrate reductase (*narG*) and copper-containing nitrite reductase (*nirK*) compared to all other treatments.

4. Discussion

4.1. Effect of amendments on soil properties and plant growth

Organic amendments and UAN impacted soil properties and plant growth. The C/N ratio was higher in compost than LD and SD, elevating

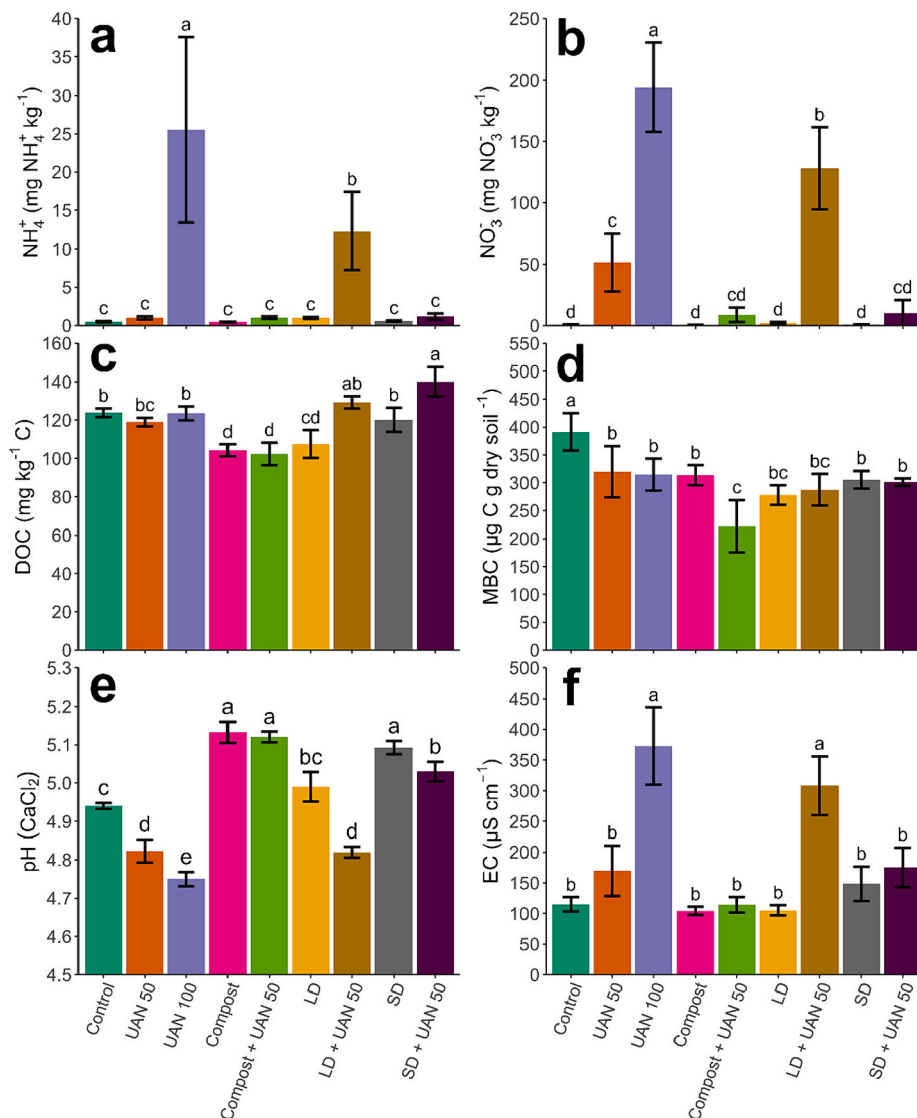


Fig. 2. Soil chemistry properties at harvest of ryegrass of nine different treatments. LD = Liquid Digestate; SD = Solid Digestate; UAN 50 = Urea Ammonium Nitrate applied at a rate of 50 kg total N ha^{-1} ; UAN 100 = Urea Ammonium Nitrate applied at a rate of 100 kg total N ha^{-1} . Bars represent mean and error bars represent standard deviation (n = 4). Tukey's HSD represents significant differences between treatments.

soil C/N ratios and causing N immobilisation as microbes utilise N to decompose C (Bolan et al., 2004). Consequently, compost's high C/N ratio resulted in less available N, producing lower shoot biomass than LD and SD. UAN had no significant effect in plant growth compared to control (Fig. 1a). Applying UAN to unamended soils initially stunted growth, likely due to the acidifying effect and reduced soil pH (CaCl₂) of 4.82 to 4.75, below the ideal >5.3 for perennial ryegrass (Fulkerson and Donaghy, 1998). Acidic soil conditions can inhibit or reduce nutrient availability, hindering plants' ability to absorb essential nutrients (such as N, P, K S). During ammonification of urea-N to ammonium-N in the UAN-amended soils, the pH increases, whilst nitrification of ammonium to nitrate N decreases the pH (Bolan et al., 2004). As NH_4^+ in UAN was likely nitrified in the early stages of plant growth making it acidic, this likely resulted in the increased mobilisation of these nutrients for the plant, and hence, UAN-amended soils recovered in the later stage of plant growth and caught up to the control. Moreover, UAN-amended soils had no additional macro-nutrient input, unlike compost, LD, and SD, which contained high levels of P, K and S (Table 1), which likely also increased plant growth as compared to UAN.

SD + UAN 50 had greater shoot biomass compared to LD + UAN 50, likely due to the higher concentration of macro and micronutrients and

relatively lower N concentration found in SD (Table 1). During drying of LD for SD production, ammonia was volatilised, reducing the total N content of SD (Table 1). As LD had a higher NH_4^+ concentration compared to SD, this may have led to soil acidification and stunted growth when UAN was applied, which likely contributed to the antagonism observed between LD and UAN.

Synergism was observed when UAN was amended with compost and SD (Table 2), likely somewhat due to the alkaline pH of the organic amendments neutralising the soil pH, reducing Al and Mn toxicity and providing increased nutrient mobility (Department of Primary Industries, 2018). Compost and SD also likely had a high pH buffering capacity due to the high C content (Latifah et al., 2018). The addition of UAN resulted in no significant difference in soil pH in compost, a slight decrease in SD, and a large decrease in LD (Fig. 2e). LD had the highest NH_4^+ levels in LD (Table 1) compared to other organic amendments. This high NH_4^+ content likely resulted in higher residual levels of NH_4^+ and NO_3^- in the soil post-harvest (Fig. 2a and b), decreasing soil pH. The beneficial application of organic matter can also increase micro-nutrient availability to plants, increasing plant growth. These micronutrients can be found in the organic amendments (Table 1), but also, the organic

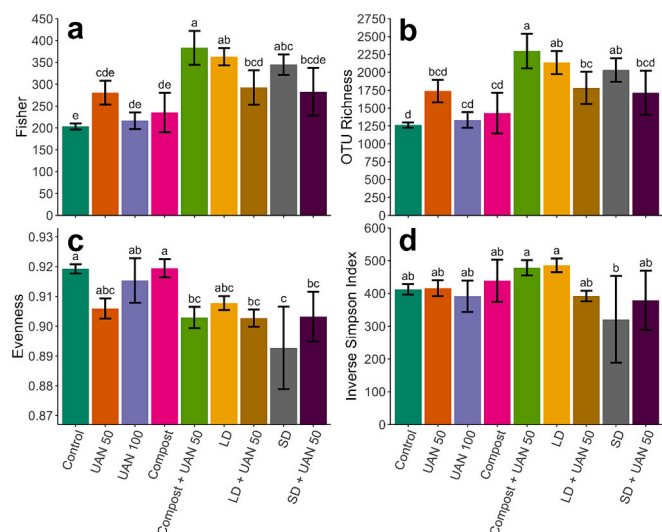


Fig. 3. Alpha diversity indexes of soil bacteria at harvest of ryegrass of nine different treatments. LD = Liquid Digestate; SD = Solid Digestate; UAN 50 = Urea Ammonium Nitrate applied at a rate of 50 kg total N ha⁻¹; UAN 100 = Urea Ammonium Nitrate applied at a rate of 100 kg total N ha⁻¹. Bars represent mean and error bars represent standard deviation (n = 4). Tukey's HSD represents significant differences between treatments.

matter can form soluble complexes within the soil, mobilising micro-nutrients (Dhaliwal et al., 2019).

The low available N of soil, compost, and SD (Table 1) resulted in N deficiency in plants and significantly increased the root to shoot ratio, as compared to all organic amendments and UAN containing treatments

(SI-Fig. 1). Such N-poor soils often stimulate root growth to optimize nutrient search (Lynch et al., 2012). Despite the available N depletion for UAN-amended compost and SD (Fig. 2a and b), initial shoot growth likely consumed most available N, reducing the root to shoot ratio. Root length, surface area, diameter, and volume (Fig. 1) of organically amended soils, with or without UAN, surpassed those in control and UAN-only treatments, reflecting increased plant growth. At harvest, these organic treatments started showing N deficiency symptoms (see SI-Figs. 8 to 13), possibly prompting root growth for nutrient mining, as observed with compost (Lynch et al., 2012). Meanwhile, UAN-amended soils displayed reduced root growth characteristics compared to control and organic amendment treatments. Organic treatments, with or without UAN, showed no significant root morphology differences.

LD had high NH₄⁺ levels and can be shown to be used as an effective N fertiliser in soils and potting media when applied appropriately (Mickan et al., 2022). The high NH₄⁺ levels in LD resulted in residual NH₄⁺ and subsequent NO₃⁻ through nitrification in soils (Beeckman et al., 2018). LD also had significantly higher N content in plant shoots (Table 3) compared to other organic amendments without UAN. Therefore, the process of LD dewatering into SD greatly reduces its available N value as seen by the reduction of NH₄⁺ in Table 1. Macro and micro-nutrients in shoots did not have an observable difference compared to control for organic amendments (Table 3). Applying organic amendments would have increased these macro- and micro-nutrients as shown by their nutrient content in Table 1. This similar nutrient content amongst control and organic amendments may be due to the higher shoot biomass, causing a 'dilution effect' (Jarrell and Beverly, 1981).

4.2. Microbial community and effect on soil and plant growth

Food waste fertilisers' addition shifted the soil microbial community and soil C and N cycling. Typically, organic amendments increase MBC

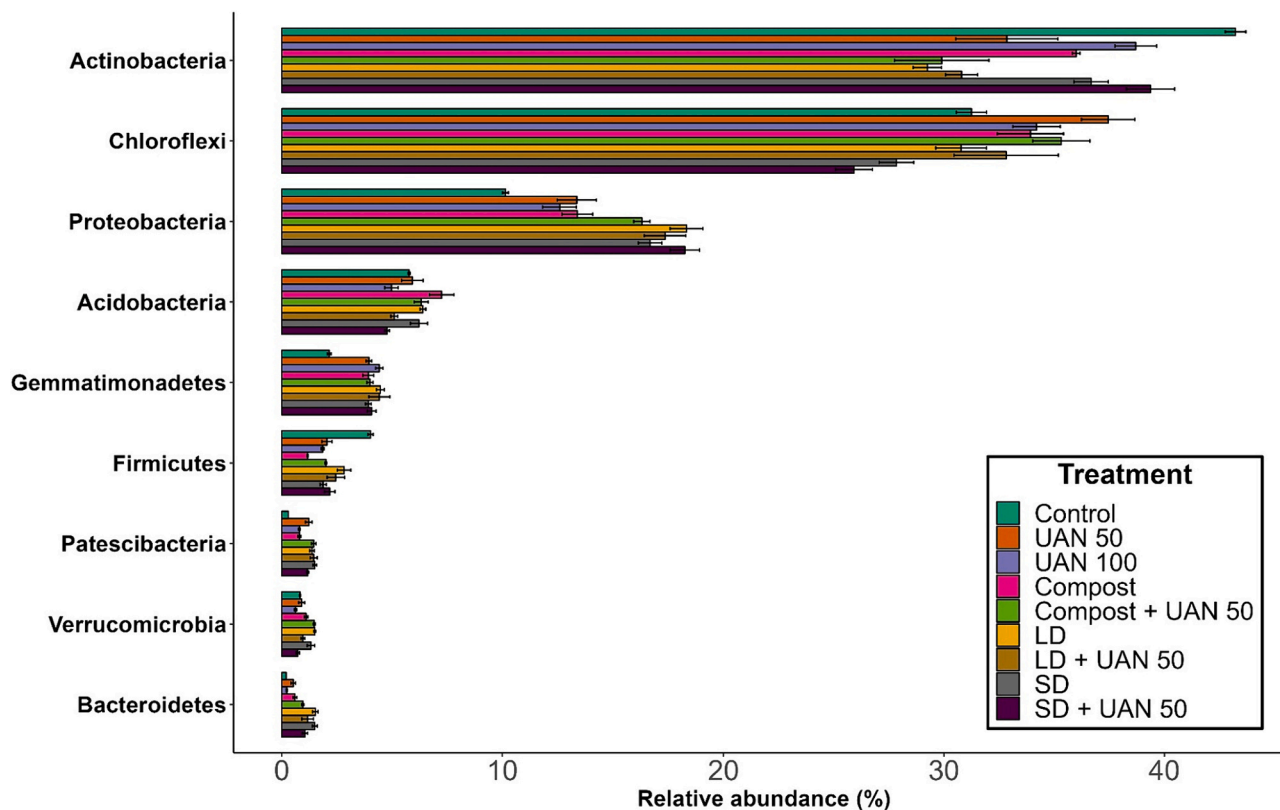


Fig. 4. Relative abundance of soil bacteria phyla at harvest of ryegrass of nine different treatments. LD = Liquid Digestate; SD = Solid Digestate; UAN 50 = Urea Ammonium Nitrate applied at a rate of 50 kg total N ha⁻¹; UAN 100 = Urea Ammonium Nitrate applied at a rate of 100 kg total N ha⁻¹; Bars represent mean and error bars represent standard deviation (n = 4).

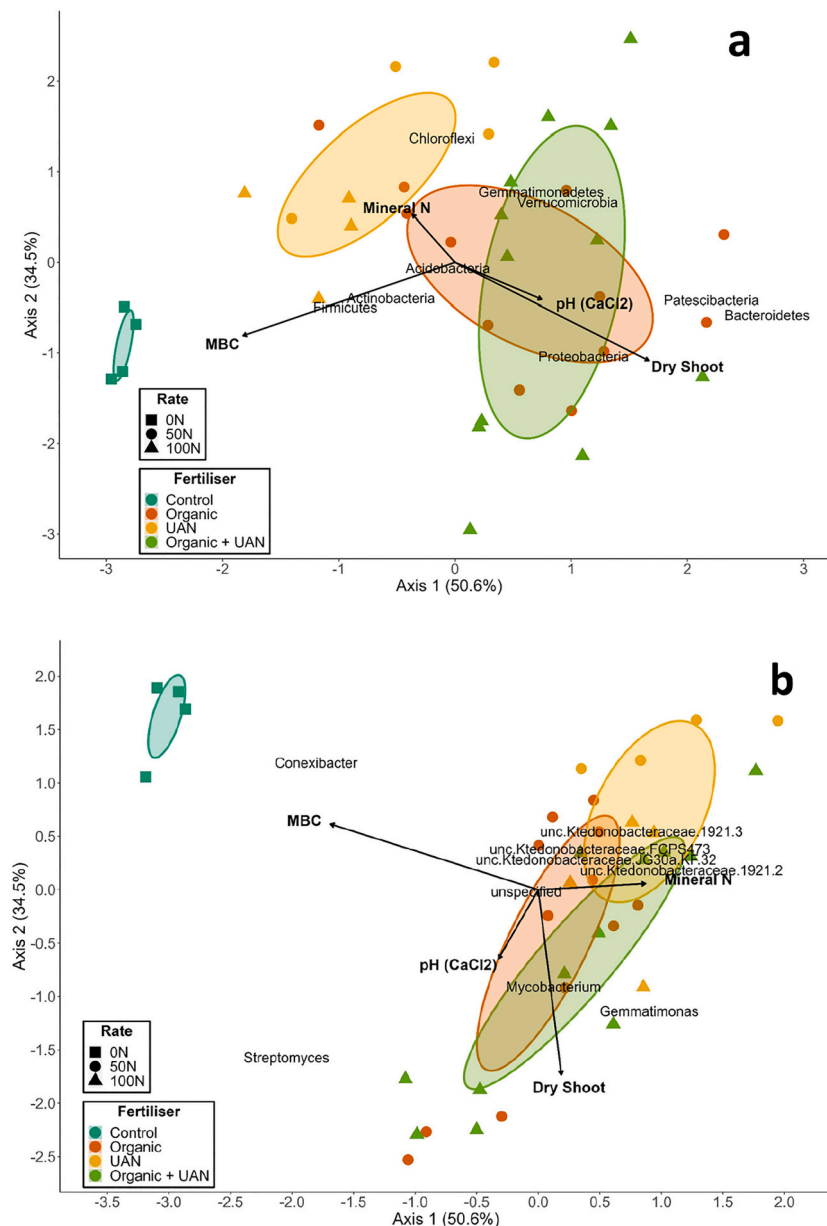


Fig. 5. Canonical correspondence analysis (CCA) biplot of phyla (a) and genera (b) showing relationships between several soil chemical parameters, plant growth parameter, fertiliser type, and rate. Arrows represent the parameters. Unc. = uncultured; LD = Liquid Digestate; SD = Solid Digestate; UAN 50 = Urea Ammonium Nitrate applied at a rate of 50 kg total N ha⁻¹; UAN 100 = Urea Ammonium Nitrate applied at a rate of 100 kg total N ha⁻¹.

(Mickan et al., 2022; Shanmugam et al., 2021), however, in this study, MBC was significantly higher in the control. This might link to the quantity and quality of amendments or increased stress conditions (low nutrients and labile C) in the control soil resulting in microbes storing more C in the body tissue rather than respiring it as CO₂ (Adingo et al., 2021). Many factors influence soil microbial diversity, such as nutrient content, C source, pH, and EC (Nannipieri et al., 2003). Organic amendments LD and SD, and amendments + UAN increased microbial diversity (Fisher's alpha and OTU richness) but decreased with soils under mineral fertilization or unamended (Fig. 3). This aligns with Liu et al. (2022) and is attributed to the organic amendments causing dominance of specific microbial guilds involved in degradation of complex organic compounds found in the LD and SD. However, Mickan et al. (2022) reported that high rates of LD decreased soil microbial diversity due to the high NH₄⁺ content. No significance difference was found in inverse Simpson diversity amongst treatments, likely due to Simpson diversity sensitivity to species evenness (DeJong, 1975).

All soils, regardless of treatment, were predominated by Actinobacteria and Chloroflexi, followed by Proteobacteria (Fig. 4). Bacteria within these phyla play a major role in soil processes and are commonly reported in agricultural soils (Chen et al., 2020). Actinobacteria have important C cycle ecophysiological roles, such as plant residue decomposition, biological N fixation, and the ability to produce antibiotics that compete for C sources and protect under certain environmental stresses (Bao et al., 2021). In addition, Actinobacteria can degrade complex compounds and recalcitrant materials such as starch, lipids, cellulose, lignins, and organic acids (Jenkins et al., 2010). Chloroflexi also typically thrive in oligotrophic environments, which explains their abundance in the nutrient-poor semi-arid soils of Western Australia (Lan et al., 2022). Further inspection of the sequence analysis revealed that oligotrophic Ktedonobacteraceae dominates Chloroflexi. Some members of this family have evolved versatile metabolic pathways including carbon monoxide oxidation and generation of energy through solar radiation and 3-hydroxypropionate bi-cycle (Klatt et al., 2013). Thus,

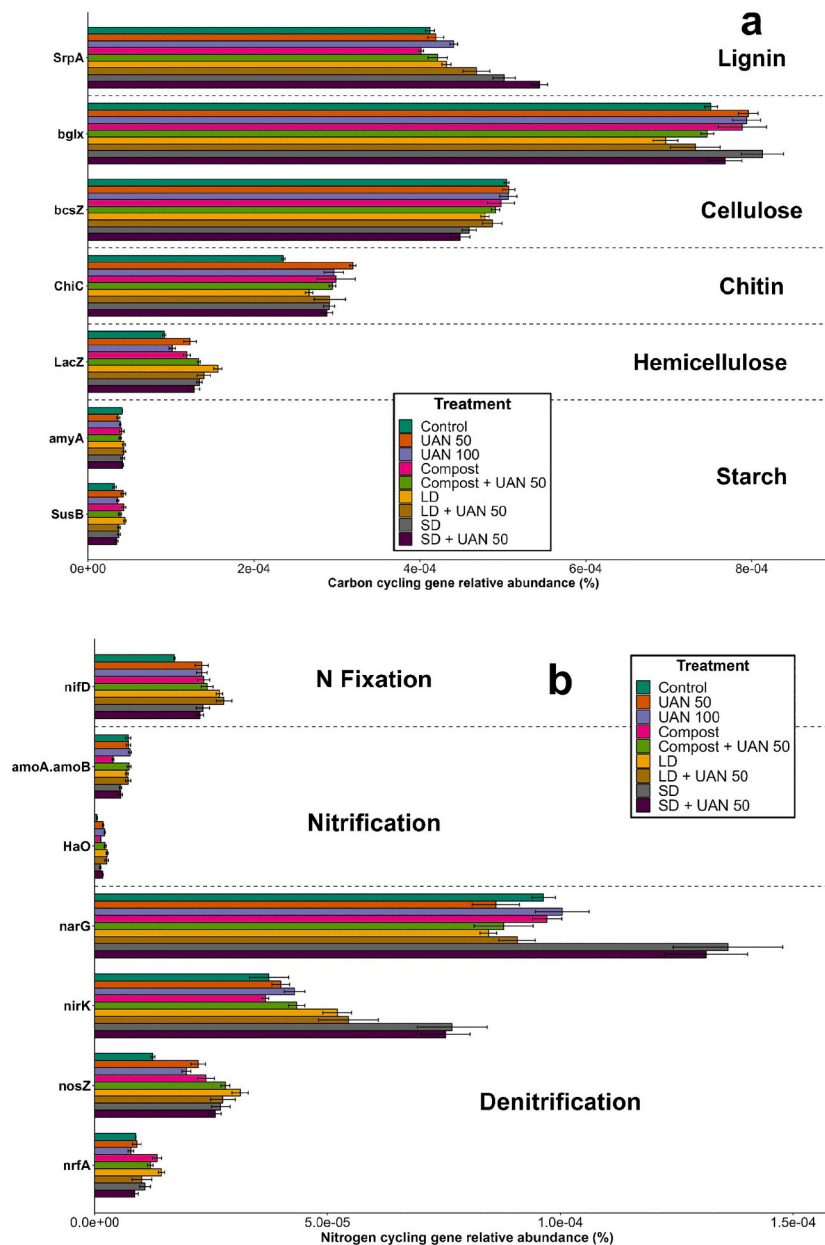


Fig. 6. PICRUSt comparison of putative carbon and nitrogen cycling gene abundances that encode specific enzymes in soil microbial communities across nine treatments after the harvest of ryegrass. Abbreviation for putative carbon cycling genes that encode specific enzymes catalase, (SrpA), beta-glucosidase (bglx), endoglucanase (bcsZ), chitinase (ChiC), beta-galactosidase (LacZ), alpha-amylase (amyA), glucoamylase (SusB). Abbreviation for putative nitrogen cycling genes that encode specific enzymes are nitrogenase (nifD), ammonia monooxygenase (amoA.amoB), hydroxylamine oxidoreductase (HaO), nitrate reductase (narG), copper-containing nitrite reductase (nirK), nitrous-oxide reductase (nosZ), nitrite reductase–ammonium forming (nrfA). LD = Liquid Digestate; SD = Solid Digestate; UAN 50 = Urea Ammonium Nitrate applied at a rate of 50 kg total N ha⁻¹; UAN 100 = Urea Ammonium Nitrate applied at a rate of 100 kg total N ha⁻¹. Bars represent mean and error bars represent standard deviation (n = 4).

Actinobacteria and Ktedonobacteraceae are oligotrophic specialists whose traits are more consistent with slow-growing K-strategists (Fontaine et al., 2003). Fig. 5a shows a marked decrease in the relative abundance of Actinobacteria in all organic amendment treatments relative to the control, showing that the soil is under environmental stress due to nutrient deficiency.

The phyla Bacteroidetes, Proteobacteria, Patescibacteria, Gemmatimonadetes, and Verrucomicrobia were most abundant in organic and organic + UAN treatments, with Bacteroidetes most responsive to organic inputs due to their fast-growing copiotrophs (Jenkins et al., 2010). Bacteroidetes was dominated by the genera *Mucilaginibacter*, *Flavisolibacter*, *Proteiniphilum* commonly found in organic materials such

as manure or compost (Mickan et al., 2022; Shanmugam et al., 2021). Bacteroidetes play important roles in P mobilisation (organic P into inorganic P) and pathogen suppression (Lidbury et al., 2021) suggesting organic amendments inputs increase their abundance via increases in soil organic P. Proteobacteria, enriched in organic C-rich soils, adeptly respond to varying C and N compounds and include metabolic specialists such as biological N fixation, denitrification, nitrification and methane oxidation (Shanmugam et al., 2021). Proteobacteria were dominated by fast growing heterotrophic r-strategist, of Rhodanobacter, *Massilia*, *Bulkholderia* and *Sphingomonas* (data not shown) (Jenkins et al., 2010). Bacteroidetes and Proteobacteria, as copiotrophic r-strategists, evolve metabolic strategies for enhanced C uptake and efficient growth,

enabling rapid consumption of labile C compounds (Griffiths et al., 1998). Thus, digestate and compost amendment promote the growth of Proteobacteria and Bacteroidetes as they are fast-growing copiotrophs that thrive in nutrient-rich environments outcompeting other slow-growing phyla.

The role of Patescibacteria remains unclear in soils (Hannula et al., 2021), but their abundance increased Patescibacteria (e.g. Saccharimonadales) with increased root biomass and C availability. Gemmatimonadetes, abundant in high-nutrient soils (Mujakić et al., 2022), aligns with organic amendments and UAN use, playing a role bioremediation and potential nutrient cycling (Liu et al., 2021). Verrucomicrobia is involved in N fixation, methane oxidation, and utilisation of complex polymers in soil for nutrient sources (Baliyarsingh et al., 2022), indicative of a K-strategist. This suggests labile C depletion by r-strategist, causing a shift to more specialised species (i.e., K-strategist). that can metabolise the remaining less-bioavailable C (Fontaine et al., 2003; Jenkins et al., 2010).

The genera of uncultured Ktedonobacteraceae were most dominant in all soils, especially those that were amended by organic amendments and UAN (Fig. 5b and SI-Fig. 6). This finding of high abundance aligns with the results reported by Megyes et al. (2021), who observed high abundance of uncultured Ktedonobacteraceae in soils amended with nitrogen, phosphorus, and potassium. However, the specific functions and roles of Ktedonobacteraceae in soil ecosystems remains to be unclear. Conexibacter were dominated in the control. Conexibacter are aerobic bacteria observed in soils with low nitrogen addition (Jien et al., 2021). Conexibacter can reduce nitrate (NO_3^-) to nitrite (NO_2^-) playing a role in nitrification as well as in soil C cycling (Chen et al., 2023; Jien et al., 2021). Gemmatimonas had high abundances in soils amended with organic amendments and UAN. Gemmatimonas are known to be found in soils enriched with carbon and nutrients and are known to suppress disease and promote plant growth (Li et al., 2017).

Further support for an increase in heterotrophic activity following digestate or compost amendment is observed in the PICRUST analysis. The increased heterotrophic activity is shown by the increase in the abundance of putative C degradation genes for chitin and hemicellulose (ChiC and LacZ) in the organic treatments compared to the control (Fig. 6a). In addition, the digestate treatments had higher abundance of putative genes involved in the degradation levels of lignin. The anaerobic digestion process is known to be challenged by lignocellulosic feedstocks due to their recalcitrant nature which often results in lignin being found in anaerobic digestate (Ren et al., 2020). This observation is consistent with similar studies by Ren et al. (2020). The application of UAN increased the abundance of putative genes involved in encoding for the decomposition of different types of carbonaceous material in soil, except for certain putative genes encoded to decompose starch (amyA) and cellulose (bcsZ). This decomposition is likely a result of increased microbial growth due to increased N availability (Ai et al., 2012).

Amendment application increased putative genes encoding N fixation (nifD), and likely reflects the high abundance the N fixer *Burkholderia* (Estrada-De Los Santos et al., 2001). Organic amendments, containing growth-promoting bacteria, boost N fixers and nifD gene presence in soil (Ndubuisi-Nnaji et al., 2020). The increase aligns with similar studies (Ren et al., 2020; Zhang et al., 2019). Interestingly, UAN increased nifD abundance too, contrasting Zhaoxiang et al. (2020) who found synthetic N fertiliser reduced nifD abundance. Despite synthetic N fertiliser reportedly reducing biological N fixation (Reinprecht et al., 2020), the reasons for this are unclear, necessitating further exploration of UAN's effect on N-fixing populations.

There was a decrease in the relative abundance of putative amoA genes in the compost- and SD-only treatment relative to the control, contradicting previous findings, such as Ren et al. (2020), where amoA. amoB gene abundance (putative genes encoded to nitrify NH_3 to NH_2OH) was similar in compost treatments than control, and Mickan et al. (2022) found putative amoA genes increase with the rate of digestate application. The decrease in amoA. amoB gene abundance in

these amendments is likely caused by NH_4^+ volatilisation during the dewatering of LD to SD (O'Connor et al., 2022). Other variables such as reduced NH_4^+ in the soil and organic N at harvest, inhibitory substances in the compost and digestate and the possibility for unknown genes that undergo ammonia oxidation (Zhang et al., 2019). Alternatively, the results might suggest that heterotrophic nitrification is the dominant pathway for SD and compost-only treatments. Previous studies indicate low pH or changes in moisture and aeration (by adding microalgae) could inhibit chemoautotrophic nitrifiers (Manzoni et al., 2012). Heterotrophic bacterial and fungal nitrifiers tend to thrive in acid soils (De Boer and Kowalchuk, 2001) and proliferate following labile C and organic N addition to soil.

Putative denitrification-related genes were considerably higher in all amended soils than control. SD had the highest gene abundance for nitrate reductase (narG; gene encoded to reduce NO_3^- to NO_2^-) and nitrite reductase (nirK; NO_2^- to NO) that produces nitric oxide (NO). This suggests an increased risk of N loss via nitrous oxide or dinitrogen in the soil is amended with SD. These findings are similar to other studies examining the effect of inorganic and organic fertilisers on denitrification (Ren et al., 2020; Zhang et al., 2019).

Limitations of molecular methods identified in this study include that PICRUST2 requires Operational Taxonomic Units (OTUs) to correspond to a defined reference sequence (Douglas et al., 2018). These reference sequences may not contain novel OTUs identified. Additionally, PICRUST2 also predicts the functional attributes of a microbiome instead of identifying them directly (Douglas et al., 2018). Other limitations of molecular methods include incomplete extraction of DNA from soil, unrepresentative soil samples, inhibition of amplification, insufficient sequencing depth and inability to identify the active pool of microorganisms (Alteio et al., 2021; Semenov, 2021). Therefore, these limitations should be considered within the results of this study.

4.3. Considerations to produce dried solid digestate

The dewatering of LD to SD has the potential to reduce handling and transportation costs associated with LD, though, the drawback of dewatering LD to SD is the NH_4^+ loss in solution via ammonia gas (O'Connor et al., 2022). The lowered NH_4^+ level in the SD did not affect plant nutrition, as there was no difference in dry shoot biomass between LD and SD-only treatments. However, if the NH_4^+ had not been volatilised during dewatering, an increased NH_4^+ concentration in the SD implies a more nutrient-dense resource, thus requiring less volume to achieve a given N fertiliser value.

The N recovery rate of SD from dewatering LD was only 32.5 %, mainly by NH_4^+ loss (>99.8 % NH_4^+ lost) (Table 1). Therefore, further processing of LD is required to either strip ammonia from digestate or lock the NH_4^+ within the liquid phase, such as through acidification to produce ammonium salts (Li et al., 2016). The acidified digestate can then be dewatered without N loss via ammonia volatilisation. A techno-economic analysis is required to determine whether acidified digestate using advanced processing facilities is economically feasible. Moreover, increased studies are required on applying acidified digestate products on soils and their subsequent plant growth. Promising studies show that plant-growth-promoting compounds (such as abscisic acid, gibberellic acid, and indoleacetic acid) can be retained when acidifying digestate (Li et al., 2016).

5. Conclusions

The study assessed the effects of food waste fertilisers (compost, LD, and SD) on soil properties, microbial community of the soil and the growth of ryegrass. LD + UAN 50 had antagonistic effects, whereas combining compost and SD with chemical fertiliser had synergistic effects on soil properties and resulted in the highest shoot biomass. The shoot biomass of SD + UAN 50 was 310 % higher than the control. The sole application of UAN to soil resulted in a significant reduction in pH that

likely stunted plant growth. LD + UAN 50 observed high residual NH_4^+ and NO_3^- levels in post-harvest with high shoot biomass. Beneficial plant growth phyla that were fast growing and consistent with r-strategists (Bacteroidetes, Proteobacteria, Patescibacteria, Gemmatimonadetes, and Verrucomicrobia) were found in higher abundances in soils applied with organic amendment and organic amendment with UAN. Putative genes encoded for N fixing were found in higher abundances in all soil amendments compared to control due to the high abundance of *Burkholderia*, a N fixer. Putative denitrification genes were more abundant in the SD samples indicating the potential loss of N via N_2O and NH_3 gases. Overall, the dewatering of LD to produce SD should be optimised as large losses of N are lost via NH_3 volatilisation. Therefore, LD pre-treatment (i.e., ammonia stripping or acidification of LD) is necessary to reduce N loss and can be a stream of revenue for food waste management companies.

CRedit authorship contribution statement

James O'Connor: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **Bede S. Mickan:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Sun K. Gurung:** Data curation, Formal analysis, Methodology, Resources, Software, Validation. **Christopher H. Bühlmann:** Conceptualization, Data curation, Methodology, Software, Validation, Visualization. **Sasha N. Jenkins:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Kadambot H.M. Siddique:** Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization. **Matthias Leopold:** Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization. **Nanthi S. Bolan:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT-GPT4 (OpenAI) in order to generate code and increase readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2024.105380>.

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