



Nutrient recovery from anaerobic digestion of food waste: impacts of digestate on plant growth and rhizosphere bacterial community composition and potential function in ryegrass

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Abstract

Global food wastage equates to about 1.3 billion tons per year, which causes serious environmental impacts. The objective of this study was to evaluate the influences of addition of digestate from food waste in comparison to a synthetic liquid urea ammonium nitrate solution on plant growth, rhizosphere bacterial community composition and diversity, and hyphal abundance of arbuscular mycorrhizal (AM) fungi. Plant and soil samples were collected at 25, 50, and 75 days after seedling emergence. Annual ryegrass growth was significantly increased by both liquid urea ammonium nitrate and digestate, and digestate was just as effective as liquid urea ammonium nitrate. Additionally, digestate (50 kg N ha⁻¹) significantly increased AM fungal hyphae density. Liquid urea ammonium nitrate (50 kg N ha⁻¹) significantly decreased AM fungal hyphae density compared with liquid urea ammonium nitrate (25 kg N ha⁻¹) at DAE 75. Digestate and liquid urea ammonium nitrate applications significantly shifted the bacterial community composition and OTU richness and changed the abundance of microbial C and N-cycling genes, while application rates had no significant effect. Structural equation modeling showed that digestate and UAN addition both directly and indirectly affected bacterial, C and N cycling genes community composition; the indirect effects were related to increased soil NO₃⁻ content and reduced pH. This study showed that the use of digestate as a soil amendment can be environmentally effective and can provide a sustainable supply of nutrients that increases soil organic C. Moreover, the use of digestate can readily be incorporated into agricultural practices with potentially less impact on soil microflora diversity and function than conventional fertilizers.

Keywords Soil microbiology · Digestate · Arbuscular mycorrhizal fungi · Functional genes · PICRUSt

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Introduction

Large quantities of food waste resulting from unused consumable food items or rejected from produce from manufacturers can have negative impacts on the environment if they are not managed adequately (Buhlmann et al. 2019). Currently, global food wastes are estimated at 1.3 billion tons per year and are expected to increase by 53% by 2025 (Adhikari et al. 2006; FAO 2011). About 97% of global food wastes ends up in landfill, where it readily decomposes to create various problems such as odor, leachate pollution of shallow and deep waters, and methane emissions (Melikoglu et al. 2013). Food waste management aims to encourage the recycling of organic waste by soil application of the waste to soil as an amendment (Tampio et al. 2016). Anaerobic digestion of various organic wastes or food for renewable energy and the production of nutrient-rich liquid and/or solid digestates are established measures that can alleviate loads on landfill while simultaneously recovering nutrients from waste resources (Zarezadeh et al. 2019).

The use of chemical fertilizers in agroecosystems can influence soil microbial communities and cause a decline in soil organic matter content (Kibblewhite et al. 2008; Liu and Greaver 2010). Specifically, the addition of N fertilizer can alter the metabolic activities of microbial communities that decompose soil C pools (Ramirez et al. 2012). For instance, Fierer et al. (2007) observed that shifts in the specific community were responsible for the decrease in decomposition rate and that an N addition decreased the relative abundance of oligotrophs that are adept at catabolizing recalcitrant C. In addition, N fertilizer addition can increase microbial N-cycling potential (Zhang et al. 2019). Furthermore, N addition can also inhibit many fungal activities through shifts in nutrient availability (Li et al. 2014a; Phillips et al. 2019), including those of arbuscular mycorrhizal (AM) fungi, which form symbiotic associations with many vascular plants and play a significant role in mediating the flow of mineral nutrients from the soil to the host plant (Phillips et al. 2019). A previous study showed that AM fungal extraradical hyphae can assimilate N mainly in the form of NH_4^+ and possibly NO_3^- and the hyphal abundance of AM fungi can be decreased under conditions of NH_4^+ supply (Zheng et al. 2014).

Utilization of digestate derived from food waste presents some advantages compared with synthetic N applications due to its plant-available nutrient content, including all of the necessary macro- and micronutrients. Moreover, digestate contains other organic elements, including some plant hormones and/or other substances not easily identifiable, which can also result in positive influences on plant growth and development (Möller et al. 2008). Application of digestate leads to higher amounts of organic C compounds and N (present as NH_4^+), which can play an important role in potentially increasing the soil C balance (Nielsen et al. 2011) and crop yield (Albuquerque et al. 2012b). In this context, the recovery of nutrients from food waste-derived digestates in agricultural systems has an important role by reducing the use of mineral fertilizers, and this leads to positive effects with respect to resource conservation and soil quality maintenance (García-Sánchez et al. 2015). Therefore, the use of digestate is an important component of integrated nutrient management for sustainable agriculture (Tampio et al. 2016). Recent findings reported that anaerobic digestates positively affect forage crop yields, especially grasses, with increases in plant growth either similar or exceeding those from equivalent amounts of traditional mineral fertilizers (Coelho et al. 2019). On the other hand, the utilization of digestate as an N source in agriculture can reduce costs (Sigurnjak et al. 2017) and environmental impacts (Möller 2015).

Soil microorganisms are central to soil ecological functioning, by providing several ecosystem services (Zeng et al. 2016). Microbial communities play a key role in organic matter transformation and in geochemical cycling, and strongly influence the soil physical characteristics as well as plant

growth (Dennis et al. 2010). Therefore, soil microbial function and activity can reflect indicators of soil quality (Andrews et al. 2004; Gianfreda and Ruggiero 2006; Stott et al. 2010; Giacometti et al. 2013; Schlöter et al. 2018); maintenance of soil functionality and diversity is essential for sustainable agricultural production. Indeed, some previous studies showed that digestate application to soil provided a source of available nutrients and had a positive impact on soil microbial community composition and diversity (García-Sánchez et al. 2015), microbial respiration, and enzyme activities (Gielnik et al. 2019). Elsewhere, only minor or non-significant soil microbial changes have been reported (Andruschkewitsch et al. 2013). In another study, application of anaerobic digestate stimulated soil bacterial growth, but not fungal growth (Walsh et al. 2012a). Food waste can contain substantial proportions of nitrogenous material which can produce high concentrations of ammonia as the waste is digested (Dai et al. 2017). Ammonia is necessary for microbial growth in anaerobic digestates, but excessive ammonia concentrations can lead to inhibition of microbial activity (Buhlmann et al. 2019).

A number of soil microbial changes have been related to functional genes associated with N cycling (Sapp et al. 2015; Zhang et al. 2019) and C transformations (Kibblewhite et al. 2008). The rhizosphere microbial community may stimulate or inhibit N and C cycling thereby influencing soil organic matter and nutrient availability to the plant (Zhu et al. 2014). The potential function of the microbial community can be estimated by quantifying the abundance of functional genes related to C and N cycling and assessing how they are impacted by agricultural management practices (Manoharan et al. 2017; Mickan et al. 2018, 2019). However, consistent predictions of how the soil microbial community responds to digestate application are difficult due to the inherent heterogeneity of both soil and digestate across studies. Therefore, consideration of a broader understanding of the dominant features of similar soil microbes and ecological theories may be useful in predicting changes in community composition following amendment (Mickan et al. 2018, 2019). For example, at the class or phylum level, gram-negative bacteria are often described as r-strategist and characterized as fast-growing with low substrate affinity (DeVries and Shade 2013). Application of digestate can decrease the relative abundance of gram-negative soil bacteria (*Planctomycetes* and *Bacteroidetes*), while the relative abundance of gram-positive (*Firmicutes*) rhizosphere bacteria exhibited the opposite trend (Caracciolo et al. 2015; Sapp et al. 2015). Following digestate application to nutrient-limited soil, slowly growing microorganisms (K-strategists) became dominant (Sapp et al. 2015). Examining ecological strategies of soil bacteria based on r-/K-selection theory can be a useful framework for predicting functional changes associated with soil bacteria following digestate application (DeVries and Shade 2013).

To date, although the effects of amendment with digestate on soil microbial characteristics have been reported, these studies have focused on digestate from manure (Albuquerque et al. 2012b; Caracciolo et al. 2015; Nölvak et al. 2016; Sigurnjak et al. 2017). The effects of different types of digestate application on soil microbial communities vary (Coelho et al. 2019). Furthermore, these studies tested only a single time point and were therefore unable to shed light on temporal dynamics of rhizosphere communities associated with digestate amendment. On the other hand, less is known about how digestate affects soil C- and N-cycling potentials, including temporal dynamics. Therefore, in our study, we evaluated how plant growth, rhizosphere bacterial community composition and function, and hyphal abundance of AM fungi were affected by digestate from food waste and liquid urea ammonium nitrate (UAN) over time in a short-term pot experiment. The aim was to provide information relevant to the use of digestate from the anaerobic processing of food waste as a substitute for mineral fertilizers (especially N-fertilizer) in sandy semiarid agricultural soil. The hypotheses were (i) that digestate would significantly influence the soil microbial community composition and function, as well as plant growth over time in comparison to liquid urea ammonium nitrate; (ii) that digestate application would increase the relative abundance of *Firmicutes* and *Actinobacteria* and enhance the potential activity of enzymes associated with C degradation and N transformations. Conversely, liquid urea ammonium nitrate application was expected to reduce the rhizosphere bacterial potential function; and (iii) that application of digestate and liquid urea ammonium nitrate together would decrease the AM fungal colonization and alter the bacterial community composition.

Materials and methods

Experimental design

A glasshouse pot experiment was established using annual ryegrass (*Lolium rigidum*), which is an important component of pastures in Australia. The experimental design consisted of five soil treatments with four replicates using a randomized block design. The soil treatments included two application rates (25 or 50 kg N ha⁻¹) of either digestate or liquid urea ammonium nitrate and an unamended (CK).

Soil collection, digestate, and liquid urea ammonium nitrate

The experiment was established using plastic pots (diameter 14.0 cm, depth 13 cm) filled with 1.6 kg of soil. The soil was collected from the top 20 cm from an agricultural farm at Katanning in south-western Australia (33°45'S, 117°27'E).

Soil was air-dried, sieved (2 mm), and mixed before use. The soil properties were exchangeable NH₄⁺-N 9.00 mg kg⁻¹, NO₃⁻-N 3.00 mg kg⁻¹, P 22.0 mg kg⁻¹, K 42.0 mg kg⁻¹, S 35.1 mg kg⁻¹, organic C 1.77%, conductivity 0.37 dS/m, and pH 5.5. The soil is a yellow sandy duplex or Typic Palexerult (USDA Soil Taxonomy). The digestate was obtained from a mesophilic anaerobic digestion facility near Perth in Western Australia, primarily treating food waste. Specifically, a 2500-m³ digester that treated mixed wastes at a hydraulic retention time of 30 days at 35 °C was used (Buhlmann et al. 2019). A detailed analysis of the nutrient composition of the digestate is shown in Table 1. The ratios of digestate application were 5 g and 10 g (fresh digestate) per 100 g dry soil (equivalent to a field application of 25 and 50 kg N ha⁻¹, respectively). This application dose was selected to avoid low inputs of organic C to soil and for keeping the N addition at an optimal rate. Fertilizer N was applied as 32% liquid urea ammonium nitrate broadcasted on the soil surface and mixed with topsoil. The liquid urea ammonium nitrate application rates were of 0.05 g and 0.1 g per 100 g soil (equivalent to a field application of 25 and 50 kg N ha⁻¹, respectively). Digestate and liquid urea ammonium nitrate were added manually and mixed thoroughly with the soil. No other fertilizers were added during the experiment period. After an equilibration phase of 10 days in the glasshouse, the plants were sown.

Seed germination and planting

Ryegrass seeds were soaked with deionized water in Petri dish until the free radicle emerged. Germinated seeds were planted

Table 1 Chemical composition of digestate

Item	Unit	Concentration
Ammonium-N	mg/L	5200
Nitrate-N	mg/L	2.00
NOx-N	mg/L	2.00
Chloride	mg/L	2500
Total phosphorus	mg/L	630
Total calcium	mg/L	2000
Total cobalt	mg/L	0.12
Total copper	mg/L	1.8
Total iron	mg/L	440
Total potassium	mg/L	1300
Total magnesium	mg/L	150
Total manganese	mg/L	7.9
Total sulfur	mg/L	160
Total nickel	mg/L	0.23
Total sodium	mg/L	980
Total zinc	mg/L	18

NOx-N nitrate + nitrite-N

at 10 mm depth in each pot, arranged in ten pairs of equal spacing. Plants were thinned to five per pot after emergence. Pots were maintained under glasshouse conditions and watered accordingly to maintain soil moisture at about 80% field capacity in the cause of the experiment. All pots were weighed daily to determine the soil water content. To evaluate the temporal dynamics of the rhizosphere microbiome, three destructive harvests for each treatment were performed at 25, 50, and 75 days after seedling emergence (DAE), representing various stages of plant growth (seedling, tillering, peak tillering). Shoot and root biomass were measured at each harvest after oven-drying at 60 °C for 72 h.

Soil analysis

At each harvest, roots were carefully removed from the bulk soil and gently shaken to remove loosely adhering soil. The more tightly adhering rhizosphere soil (Mickan et al. 2018) was collected and stored at 4 °C for soil measurements and –20 °C for genomic DNA extraction.

The electrical conductivity (EC) and pH were measured using a probe inserted into water mixtures (1:5 soil/water ratio). Total soil C (TC) and N (TN) were measured using an Elementar Analyser (Vario Macro CNS, Elementar, Germany). Dissolved organic C was extracted using a combination of 20 g soil to 80 mL 0.5 M K₂SO₄ and analyzed using an OI Analytical Aurora 1030 Wet Oxidation TOC Analyzer (College Station, TX, USA). The same mixture (20 g with 80 mL 0.5 M K₂SO₄) was used for measuring the soil N-nitrate (NO₃[–]-N) and the soil exchangeable ammonium-N (NH₄⁺-N), the content of exchangeable NH₄⁺ was measured using the salicylate–nitroprusside method (Searle 1984) and NO₃[–] concentration using the hydrazine reduction method (Kempers and Luft 1988) on an automated flow injection Skalar AutoAnalyser (San plus, Skalar Analytical, The Netherlands).

AM fungal root colonization and extraradical hyphal length assessment

At each harvest, roots were carefully washed free of soil, and a subsample of known weight (0.5 g fresh weight) was taken from each root sample. Root sub-samples were cut into 1 cm pieces and cleared in 10% KOH, acidified, and stained with Trypan blue (0.05%) in lactoglycerol (1:1:1 lactic acid/glycerol/water) for AM fungal quantification (Abbott and Robson 1981). The percentage of AM fungal structures was determined microscopically at 200× magnification with the gridline intersect method (Giovannetti and Mosse 1980). Soil cores (1-cm diameter) were taken from the center of the pots to a depth of 7 cm. All soil cores were stored at 5 °C for hyphal extraction. Hyphal length in the soil was measured as described by Jakobsen et al. (1992).

DNA extraction, PCR amplification, and sequencing

DNA was extracted from 0.4 g of rhizosphere soil using a Power Soil® DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) and following the protocol of the manufacturer. Extracted DNA was quantified (Qubit, Life Technologies, Australia) and adjusted to 1 ng/μL using molecular-grade water and stored at –20 °C until further analysis. The DNA preparation and sequencing library preparation were performed following the recommendations described by Scholer et al. (2017) and Vestergaard et al. (2017). Amplification of the target 16S rRNA genes was carried out following the protocol of Mickan et al. (2018) using 27F/519R bacterial primers (Caporaso et al. 2010) amended with the barcodes of Golay (Caporaso et al. 2012) with negative controls.

Bioinformatics and PICRUST

DNA sequencing was on the Illumina Mi-seq platform. Paired-end reads were assembled by aligning the forward and reverse reads using PEAR (version 0.9.5) (Zhang et al. 2014). The primers were identified, and using Quantitative Insights into Microbial Ecology (QIIME 1.8) (Caporaso et al. 2010) USEARCH (version 8.0.1623, Edgar et al. 2011) and UPARSE software, the trimmed sequences were processed. Using the USEARCH, sequences were denoized, quality filtered, and chimera checked according to abundance. The reads were mapped back to the operational taxonomic units (OTUs) based on 97% identity to obtain the number of reads in each OTU. Using the Greengenes database5 to assign the QIIME taxonomy (version 13_8, Aug 2013), a Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) (<https://picrust.github.com>) was performed (Langille et al. 2013). The genes characterized were those identified in C and N cycling (Mickan et al. 2018). The metagenomes were collapsed into the Kyoto Encyclopedia of Genes and Genomes. For unknown reasons, MiSeq sequencing of one of the samples belonging to digestate (25 kg N ha^{–1}) at the second sampling and one of the samples belonging to digestate (50 kg N ha^{–1}) and one of the samples belonging to liquid urea ammonium nitrate (50 kg N ha^{–1}) at the third sampling failed. Thus, we removed these three samples in the analysis.

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the effect of digestate and liquid urea ammonium nitrate application on plant growth, mycorrhizal parameters, soil parameters, bacterial community composition, and relative abundance, with significant results further analyzed with post hoc test for multiple comparisons. Bray–Curtis dissimilarity was used to analyze bacterial community compositional changes at OTU

level, and the first axis on non-metric multidimensional scaling (NMDS1) was used in subsequent structural equation modeling (SEM) analysis. The significance of different fertilizer driving bacteria community composition was assessed with permutational multivariate analysis of variance (PERMANOVA) using distance matrices (adonis function) and square root-transformed OTU relative abundance data. All data analyses were conducted in the R statistical environment. Structural equation modeling was carried out to test for directly and indirectly relationships among observed factors. In our study, SEM analysis was used to gain an understanding of how digestate and urea application mediate alterations in AM fungal colonization and bacterial diversity and composition, and then affects the plant biomass. In SEM analysis, the data were fitted to the models using the maximum likelihood estimation method. Each variable has a relative contribution degree shown in arrow in path diagram. Path coefficients and explained variability are estimated in models. Adequate model fits were indicated by the χ^2 test ($df > 5$; $P > 0.05$) and a low RMSEA ($P < 0.05$).

Results

Changes in soil properties after fertilization

The soil physicochemical properties at three harvest times are shown in Table 2. Soil pH markedly decreased with the increase in fertilizer application rate. Inorganic N (exchangeable NH_4^+ and NO_3^-) and dissolved organic C were correlated with the sampling period (Table 2). Both dissolved organic C and exchangeable NH_4^+ -N content increased over time. The exchangeable NH_4^+ -N content was higher at DAE 75 and ranged from 27.6 to 32.7 mg kg^{-1} and DOC ranged from 34.9 to 53.6 g kg^{-1} , across the treatments. Exchangeable NH_4^+ -N was significantly higher in liquid urea ammonium nitrate treatments at DAE 75 than in the digestate and control treatments, while there were no significant differences between fertilization rates. DOC in digestate (50 kg N ha^{-1}) was 6.26% and 29.1% higher at DAE 25 and DAE 75, respectively, compared with digestate (25 kg N ha^{-1}). However, DOC for liquid urea ammonium nitrate (50 kg N ha^{-1}) was 10.2% and 27.6% lower at DAE 50 and DAE 75 compared with liquid urea ammonium nitrate (25 kg N ha^{-1}). In addition, NO_3^- -N content was higher at DAE 25, decreasing over time. Neither digestate nor liquid urea ammonium nitrate application significantly impacted soil total organic C and N.

Plant growth, AM fungal colonization, and extraradical hyphal length

Dry shoot biomass significantly varied with harvest time (Fig. 1a). Based on an average of the two amendment rates, shoot biomass in digestate and liquid urea ammonium nitrate

were significantly increased by 51% and 42% at DAE 25, 68% and 69% at DAE 50, and 74% and 75% at DAE 75, respectively (Fig. 1a). Shoot biomass in the higher rates of both digestate (50 kg N ha^{-1}) and liquid urea ammonium nitrate (50 kg N ha^{-1}) was 28% and 23% more than those of their respective lower rates (25 kg N ha^{-1}) at DAE 50, and 44% and 38% more at DAE 75 (Fig. 1a). Similarly, root biomass varied with harvest time (Fig. 1b). Across the two amendment rates, root biomass in digestate and liquid urea ammonium nitrate increased by 55% and 58% at DAE 50 and by 57% and 61% at DAE 75, respectively (Fig. 1b). Root biomass in the higher rates of both digestate (50 kg N ha^{-1}) and liquid urea ammonium nitrate (50 kg N ha^{-1}) were 18% and 18% more than those of their respective lower rates (25 kg N ha^{-1}) at DAE 75.

By DAE 50, liquid urea ammonium nitrate (50 kg N ha^{-1}) had significantly decreased the AM fungal colonization by 23%, compared with liquid urea ammonium nitrate (25 kg N ha^{-1}) (Fig. 1c). However, neither digestate nor liquid urea ammonium nitrate application affected AM fungal colonization at DAE 75 (Fig. 1c). At DAE 50, hyphal length did not respond to either digestate or liquid urea ammonium nitrate application (Fig. 1d). At DAE 75, hyphal length in the liquid urea ammonium nitrate at 50 kg N ha^{-1} treatment was significantly decreased by 31% compared with liquid urea ammonium nitrate at 25 kg N ha^{-1} , while there were no significant differences between liquid urea ammonium nitrate at 50 kg N ha^{-1} and the control treatment (Fig. 1d). Hyphal length following application of digestate at 50 kg N ha^{-1} was significantly increased (by 29%), whereas digestate amendment rates had no effect on hyphal length (Fig. 1d).

Relative abundance of bacterial phyla

In order to visualize the overall distribution of OTUs at the 97% similarity level, a non-metric multidimensional scaling (NMDS) plot showed that the bacterial rhizosphere community composition was influenced by amendment treatments (Fig. 3). The separation between different amendment treatments indicates that the bacterial rhizosphere communities are dissimilar under these conditions. Subsequent community analysis by PERMANOVA showed that bacterial rhizosphere community composition was influenced mostly by different fertilizer (DAE 25, $P = 0.019$; DAE 50, $P < 0.001$; DAE 75: $P < 0.001$), but not by application rate (Table 3). In addition, the bacterial community composition was influenced by fertilizer ($P < 0.001$) and harvest time ($P < 0.001$) (Fig. S4c).

All fertilizer applications strongly affected microbial composition. As shown in Fig. 2, *Proteobacteria* was the most abundant bacterial phylum across the treatments, accounting for 31, 29, and 29% of all taxa on average at DAE 25, DAE 50, and DAE 75, followed by *Firmicutes* (18, 18, and 17%), *Acidobacteria* (14, 15, and 14%), *Bacteroidetes* (8.9, 7.5, and

Table 2 Exchangeable NH_4^+ -N, NO_3^- -N, dissolved organic C (DOC), pH, soil electrical conductivity, total C and N in rhizosphere soil of annual ryegrass harvested at 25, 50, and 75 days after emergence (DAE) for the well-watered condition. CK: control; Diges_25: digestate, 25 kg N ha^{-1} ;

Diges_50: digestate, 50 kg N ha^{-1} ; UAN_25: liquid urea ammonium nitrate, 25 kg N ha^{-1} ; UAN_50: liquid urea ammonium nitrate, 50 kg N ha^{-1} . Data are means \pm standard errors ($n = 4$). For each parameter, data followed with the same letter are not significantly different ($P < 0.05$)

		Exchangeable NH_4^+ -N (mg kg^{-1})	NO_3^- -N (mg kg^{-1})	DOC (g kg^{-1})	pH	EC ($\mu\text{S cm}^{-1}$)	TC (%)	TN (%)
DAE 25	CK	24.6 \pm 1.10 b	2.48 \pm 0.12 c	0.33 \pm 0.01 b	5.45 \pm 0.01 a	474 \pm 76.8	1.67 \pm 0.11	0.15 \pm 0.01
	Diges_25	25.6 \pm 2.94 b	7.78 \pm 0.49 c	0.37 \pm 0.01 a	5.49 \pm 0.05 a	520 \pm 59.6	1.58 \pm 0.06	0.17 \pm 0.01
	UAN_25	21.7 \pm 2.06 b	15.4 \pm 4.46 c	0.34 \pm 0.01 ab	5.46 \pm 0.02 a	574 \pm 62.4	1.65 \pm 0.04	0.17 \pm 0.01
	Diges_50	29.6 \pm 1.23 ab	55.7 \pm 9.24 b	0.37 \pm 0.02 a	5.39 \pm 0.07 ab	630 \pm 67.5	1.57 \pm 0.08	0.16 \pm 0.03
	UAN_50	36.2 \pm 3.98 a	85.5 \pm 3.76 a	0.33 \pm 0.01 b	5.30 \pm 0.02 b	661 \pm 67.9	1.55 \pm 0.04	0.21 \pm 0.01
DAE 50	CK	14.1 \pm 0.47 ab	1.79 \pm 0.11 b	0.33 \pm 0.00 a	5.51 \pm 0.04 a	572 \pm 10.9 b	1.93 \pm 0.16	0.14 \pm 0.02 b
	Diges_25	14.9 \pm 0.77 ab	2.11 \pm 0.11 ab	0.31 \pm 0.01 b	5.51 \pm 0.01 a	626 \pm 49.7 ab	1.79 \pm 0.22	0.14 \pm 0.01 b
	UAN_25	12.8 \pm 0.95 c	2.06 \pm 0.23 ab	0.33 \pm 0.01 a	5.46 \pm 0.01 ab	627 \pm 15.0 ab	1.89 \pm 0.06	0.14 \pm 0.01 b
	Diges_50	15.5 \pm 0.25 a	2.27 \pm 0.05 a	0.33 \pm 0.01 a	5.41 \pm 0.02 b	671 \pm 11.1 a	1.70 \pm 0.05	0.12 \pm 0.01 b
	UAN_50	15.0 \pm 0.70 ab	2.33 \pm 0.08 a	0.30 \pm 0.01 b	5.33 \pm 0.04 c	603 \pm 9.68 ab	1.95 \pm 0.08	0.22 \pm 0.02 a
DAE 75	CK	25.3 \pm 0.76 b	0.36 \pm 0.01 c	0.44 \pm 0.01 b	5.60 \pm 0.01 a	705 \pm 43.5	1.68 \pm 0.09	0.16 \pm 0.01 bc
	Diges_25	27.3 \pm 1.17 b	0.41 \pm 0.03 c	0.48 \pm 0.01 b	5.50 \pm 0.01 b	695 \pm 70.0	1.47 \pm 0.08	0.15 \pm 0.01 bc
	UAN_25	40.4 \pm 1.63 a	0.54 \pm 0.02 ab	0.63 \pm 0.05 a	5.46 \pm 0.00 c	706 \pm 80.2	1.83 \pm 0.20	0.17 \pm 0.01 b
	Diges_50	28.3 \pm 0.99 b	0.49 \pm 0.02 b	0.67 \pm 0.04 a	5.42 \pm 0.00 d	703 \pm 63.3	1.71 \pm 0.17	0.13 \pm 0.01 c
	UAN_50	42.2 \pm 1.63 a	0.58 \pm 0.01 a	0.46 \pm 0.02 b	5.41 \pm 0.01 d	771 \pm 42.1	1.64 \pm 0.09	0.21 \pm 0.01 a

Fig. 1 Total shoot dry mass (a), root dry mass (b), AM fungal colonization (c), and hyphal length in soil (d) of annual ryegrass assessed at 25, 50, and 75 days after emergence (DAE). DAE 25, DAE 50, DAE 75 refer to 25, 50, and 75 days of annual ryegrass after emergence. CK: control; Diges_25: digestate, 25 kg N ha^{-1} ; Diges_50: digestate, 50 kg N ha^{-1} ; UAN_25: liquid urea ammonium nitrate, 25 kg N ha^{-1} ; UAN_50: liquid urea ammonium nitrate, 50 kg N ha^{-1} . Error bars show standard errors of the mean

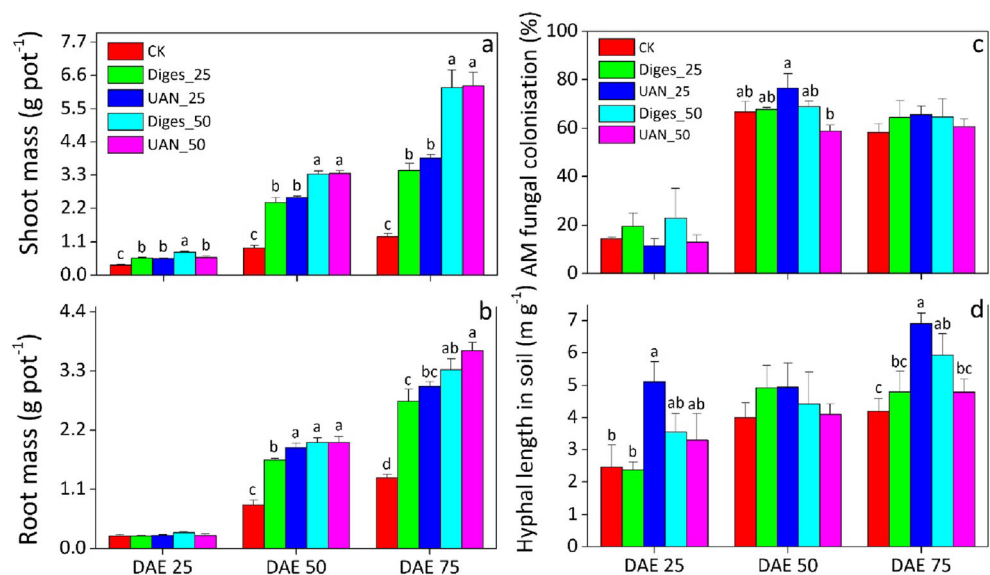


Table 3 OTU community assemblage analysis by PERMANOVA results based on 97% similarity OTU abundance data (square root transformed), using 999 permutations. Treatments consisted of fertilizer (digestate and UAN) and application rate (0, 25 and 50 kg N ha⁻¹). DAE

25, DAE 50, DAE 75 refer to 25, 50, and 75 days of annual ryegrass after emergence. UAN: liquid urea ammonium nitrate. Significant *P* values indicated in *italics*

		Degrees of freedom	Sum of squares	Mean squares	F.model	<i>R</i> ²	Pr (>F)
DAE 25	Fertilizer	2	0.55	0.27	1.89	0.18	<i>0.019</i>
	Rate	1	0.15	0.15	1.06	0.05	0.328
	Fertilizer×rate	1	0.14	0.14	0.98	0.05	0.391
	Residuals	15	2.17	0.14	—	0.72	—
	Total	19	3.01	—	—	1.00	—
DAE 50	Fertilizer	2	0.43	0.21	4.35	0.34	<i>< 0.001</i>
	Rate	1	0.09	0.09	1.78	0.07	0.066
	Fertilizer×rate	1	0.07	0.07	1.35	0.05	0.142
	Residuals	14	0.69	0.05	—	0.54	—
	Total	19	1.28	—	—	1.00	—
DAE 75	Fertilizer	2	0.28	0.14	2.84	0.27	<i>< 0.001</i>
	Rate	1	0.06	0.06	1.19	0.06	0.205
	Fertilizer×rate	1	0.05	0.05	0.97	0.05	0.391
	Residuals	13	0.64	0.05	—	0.62	—
	Total	19	1.03	—	—	1.00	—

7.9%) and *Actinobacteria* (8.8, 8.8, and 8.3%), but other taxa were present at lower abundance. Application of liquid urea ammonium nitrate decreased the abundance of *Acidobacteria* and *Planctomycetes* by 31.2 and 17.8% (DAE 25), 18.0 and 15.2% (DAE 50), and 20.8 and 22.3% (DAE 75), respectively, compared with no fertilizer for the average of the two amendments. Application of liquid urea ammonium nitrate increased the abundance of *Firmicutes* and *Cyanobacteria* by 29.0 and 39.1%, respectively, at DAE 75. Digestate

significantly increased the relative abundance of *Firmicutes* and *Bacteroidetes* by 10.0 and 22.7%, respectively, at DAE 25 for the average of the two rates (Fig. 3). However, digestate amendment did not affect the relative abundance of *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi* *Planctomycetes*, and *Verrucomicrobia* at DAE 75 (Fig. 2). At the genus level, the relative abundance of *Bacillus* belonging to the phylum *Firmicutes* was the highest, accounting for 8.2, 9.5, and 9.3% of the bacterial composition

Fig. 2 Rhizosphere bacterial relative abundance observed at the Phylum level of annual ryegrass assessed at 25, 50, and 75 days after emergence (DAE). The group accounting for ≥ 1% are shown while those < 1% are integrated into ‘other’. See Fig. 1 for treatment descriptions. Error bars are the standard error of the mean

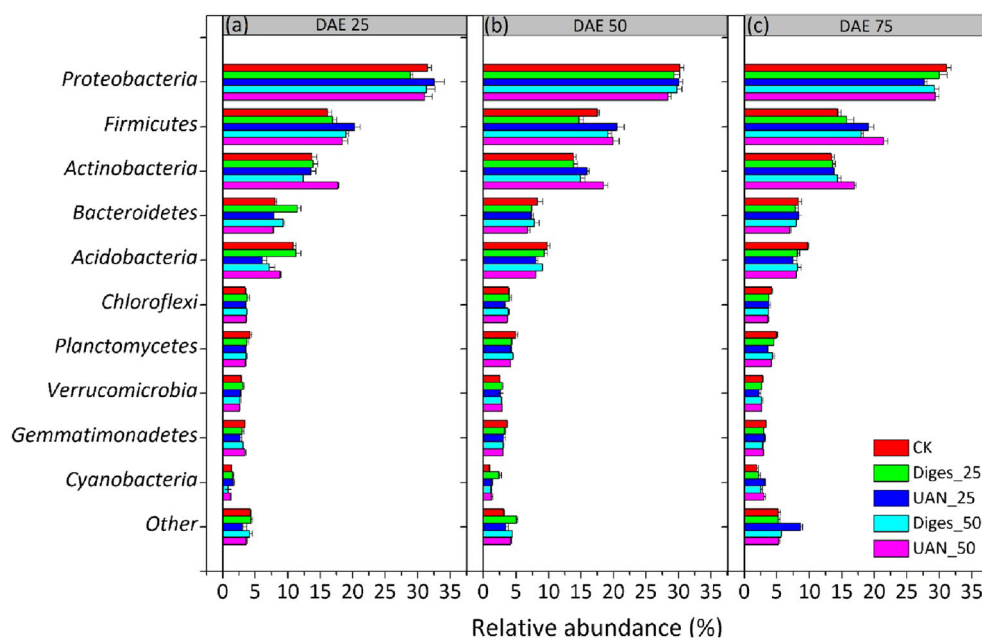
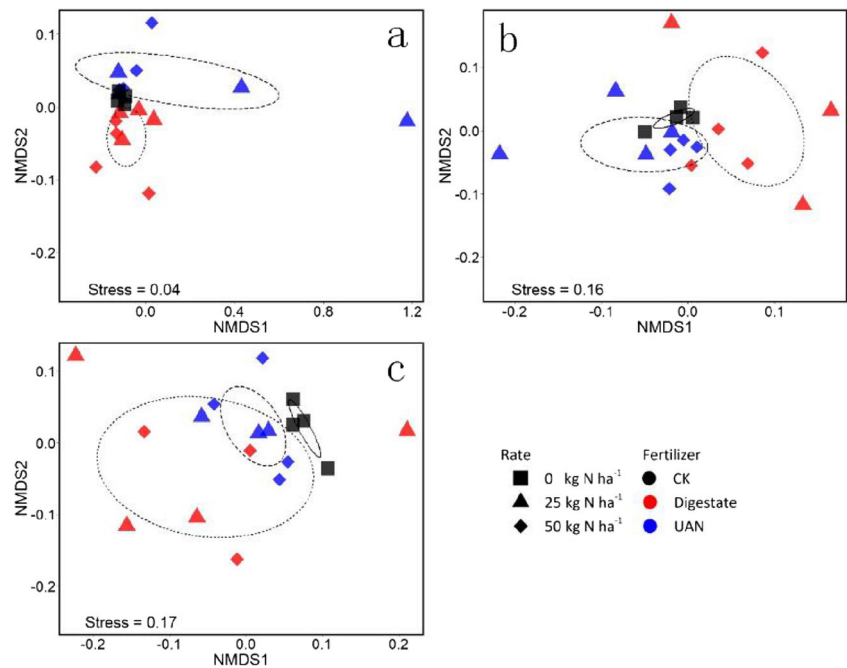


Fig. 3 Non-metric multidimensional scaling plot of soil bacterial communities of annual ryegrass assessed at 25 (a), 50 (b), and 75 (c) days after emergence (DAE) using OTU based (97% similarity) Bray–Curtis dissimilarities distance. See Fig. 1 for treatment descriptions



on average at DAE 25, DAE 50, and DAE 75, followed by *Chitinophagaceae* (3.7, 4.2, and 4.4%), *Kaistobacter* (2.8, 2.8, and 2.6%), and *Rhodoplanes* (1.7, 1.9, and 2.1%), but other taxa were present at lower abundance. The relative abundance of the *Bacillus*, *Rhodococcus*, and *Streptomyces* were greater in liquid urea ammonium nitrate (50 kg N ha⁻¹) compared with those in other treatments at DAE 25 and 50 (Tables S2–S4). In addition, liquid urea ammonium nitrate (50 kg N ha⁻¹) and digestate (50 kg N ha⁻¹) significantly decreased the relative abundance of *Rhodoplane*, compared with their respective lower rate (Tables S2–S4). In contrast, soil application of liquid urea ammonium nitrate (50 kg N ha⁻¹) and digestate (50 kg N ha⁻¹) resulted in a decrease in the relative abundance of *Alicyclobacillus*.

Bacterial OTU richness and diversity

The OTU richness and phylogenetic diversity of rhizosphere soil were influenced by digestate and liquid urea ammonium nitrate (Table 4). Although the rarefaction curves did not plateau, a high proportion of the bacterial population was sampled as inferred by the Good's coverage estimator score reaching 90 to 96%, 94 to 98%, and 87 to 98% coverage at DAE 25, 50, and 70, respectively. Comparing with lower application rates, bacterial OTU richness significantly increased with increasing amount of digestate addition, while liquid urea ammonium nitrate exhibited the opposite trend at DAE 75. By DAE 50, the digestate (50 kg N ha⁻¹) significantly increased the Shannon index, compared with the lower rate. However, no significant differences among fertilizer treatments were observed for rhizosphere Shannon index at DAE

25 and 75 (Table 4). Furthermore, neither digestate nor liquid urea ammonium nitrate application significantly impacted evenness (Table 4).

Rhizosphere bacterial metabolic profile prediction: PICRUST

We used the PICRUST program to predict metagenome functional content based on the Kyoto encyclopedia of genes and genomes (KEGG) classification. The nearest sequenced taxon index (NSTI) was used to quantify the availability of nearby genome representatives for each sample. The accuracy of PICRUST decreased with increases in NSTI scores. The average NSTI for all samples for the metagenomic predictions was 0.128 ± 0.001 in this study. Our NSTI values are lower than those of previous work (Langille et al. 2013), which revealed higher NSTI values in environmental communities (0.17 ± 0.02). Additionally, there was low variability among all samples (Table S5) and these data were comparable to other soil experiments (Chen et al. 2016).

Putative rhizosphere C cycling genes Predicted rhizosphere bacterial functional genes responsible for degradation of starch, hemicellulose, cellulose, chitin, and lignin were detected in all rhizosphere soil samples (Fig. 4). Overall, liquid urea ammonium nitrate significantly decreased the predicted abundance of genes coding for beta-galactosidase involved in hemicellulose degradation. At DAE 25, digestate (50 kg N ha⁻¹) significantly decreased the predicted abundance of genes coding for alpha-amylase and glucoamylase involved in starch degradation, xylanase

Table 4 Alpha diversity indices based at the OTU resolution (similarity = 97%) of rhizosphere soil bacteria following different fertilizer treatments of annual ryegrass assessed at 25, 50, and 75 days after emergence (DAE). CK: control, 0 kg N ha⁻¹; Diges_25: digestate, 25 kg N ha⁻¹; Diges_50: digestate, 50 kg N ha⁻¹; UAN_25: liquid urea ammonium nitrate, 25 kg N ha⁻¹; UAN_50: liquid urea ammonium nitrate, 50 kg N ha⁻¹. Data are means ± standard errors

Time	Treatment	Shannon index	OTU richness	Inverse Simpson	Goods coverage	Evenness
DAE 25	CK	7.06 ± 0.02 a	4477 ± 293 a	337 ± 9.39	0.96 ± 0.01	0.86 ± 0.01
	Diges_25	6.87 ± 0.02 ab	2955 ± 206 bc	332 ± 34.7	0.92 ± 0.02	0.85 ± 0.02
	UAN_25	6.80 ± 0.17 ab	2038 ± 495 c	262 ± 13.8	0.90 ± 0.02	0.82 ± 0.03
	Diges_50	6.76 ± 0.04 b	3512 ± 18.6 b	308 ± 39.2	0.92 ± 0.02	0.86 ± 0.01
	UAN_50	6.95 ± 0.03 ab	2830 ± 223 bc	265 ± 13.8	0.91 ± 0.02	0.85 ± 0.01
DAE 50	CK	7.15 ± 0.01 a	5167 ± 91.4 a	352 ± 9.38 a	0.98 ± 0.00 a	0.85 ± 0.01
	Diges_25	7.04 ± 0.04 b	3992 ± 75.6 c	398 ± 2.11 a	0.94 ± 0.02 c	0.85 ± 0.00
	UAN_25	7.08 ± 0.02 ab	4287 ± 39.3 b	223 ± 30.1 c	0.96 ± 0.00 ab	0.84 ± 0.01
	Diges_50	7.18 ± 0.02 a	4674 ± 93.3 b	351 ± 7.30 ab	0.95 ± 0.00 c	0.84 ± 0.00
	UAN_50	6.85 ± 0.10 b	4235 ± 247 b	315 ± 15.4 b	0.96 ± 0.00 ab	0.84 ± 0.00
DAE 75	CK	7.09 ± 0.05	4829 ± 91.1 ab	328 ± 27.3	0.98 ± 0.00	0.85 ± 0.01
	Diges_25	7.05 ± 0.03	3753 ± 68.9 c	335 ± 35.2	0.96 ± 0.00	0.85 ± 0.01
	UAN_25	7.12 ± 0.01	5071 ± 253 a	282 ± 11.3	0.87 ± 0.11	0.84 ± 0.01
	Diges_50	7.11 ± 0.04	4239 ± 195 b	297 ± 51.4	0.96 ± 0.00	0.83 ± 0.01
	UAN_50	7.10 ± 0.01	4580 ± 173 b	310 ± 6.33	0.97 ± 0.00	0.84 ± 0.01

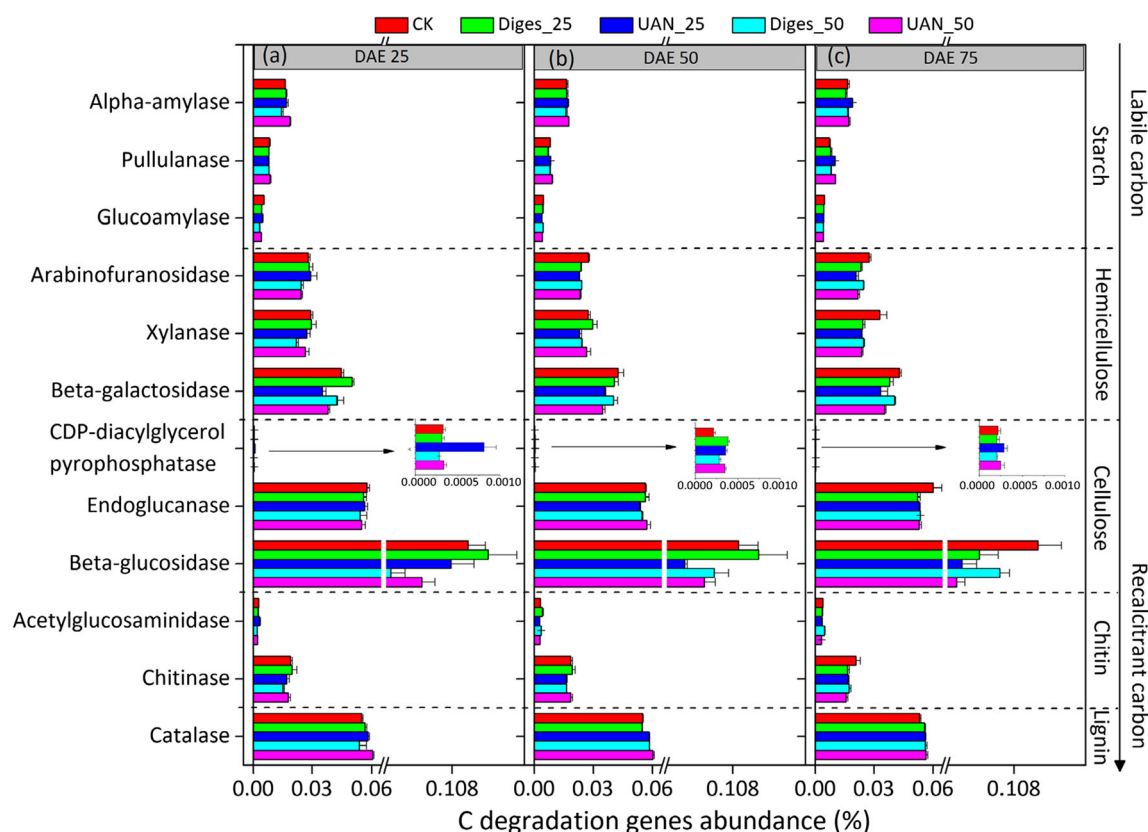


Fig. 4 Rhizosphere PICRUSt C degrading gene abundance of annual ryegrass assessed at 25, 50, and 75 days after emergence (DAE). See Fig. 1 for treatment descriptions. Error bars are the standard error of the mean

for hemicellulose degradation, and beta-glucosidase for cellulose degradation, compared with the liquid urea ammonium nitrate and control treatments (Fig. 4a). The abundances of predicted genes involved in starch, hemicellulose, cellulose, pectin, and chitin degradation were enhanced with increasing liquid urea ammonium nitrate application at DAE 50 (Fig. 4b). At DAE 75, digestate significantly decreased the predicted abundance of genes coding for alpha-amylase and pullulanase involved in starch degradation, in comparison with the liquid urea ammonium nitrate treatments. However, liquid urea ammonium nitrate significantly decreased the abundance of putative genes associated with arabinofuranosidase and xylanase involved in hemicellulose degradation, endoglucanase, and beta-glucosidase for cellulose degradation and chitinase for chitin degradation. The abundances of putative genes involved in lignin degradation were enhanced by applications of digestate and liquid urea ammonium nitrate fertilization. Most of the detected rhizosphere putative genes were not significantly affected by digestate fertilization (Fig. 4c).

Putative rhizosphere N cycling genes Overall, digestate and liquid urea ammonium nitrate addition increased the predicted gene abundance of *amoA*, *amoB*, and *Hao* involved in nitrification, *nifH* involved in N₂ fixation as compared with CK. However, compared with liquid urea ammonium nitrate (50 kg N ha⁻¹), digestate addition (at both rates) significantly decreased *amoA*, *amoB*, and *Hao* abundance (Fig. 5). At DAE 25, the application of liquid urea ammonium nitrate at both rates significantly decreased predicted genes (*nosZ* and *nirS*) involved in denitrification. While predicted gene abundance of *nosZ* involved in denitrification and *nirA*, *narB* involved in assimilatory N reduction significantly decreased with increasing digestate addition rate (Fig. 5a). The liquid urea ammonium nitrate treatments enhanced the predicted gene abundances of *nrfA* and *nrfH* for dissimilatory N reduction, and *ureC* involved in urea hydrolysis at DAE 50 and 75, and reduced *narB* for assimilatory N reduction at DAE 50, *nirS* for denitrification at DAE 75. The application of digestate did not significantly alter most predicted genes involved in N cycling at DAE 75 (Fig. 5c).

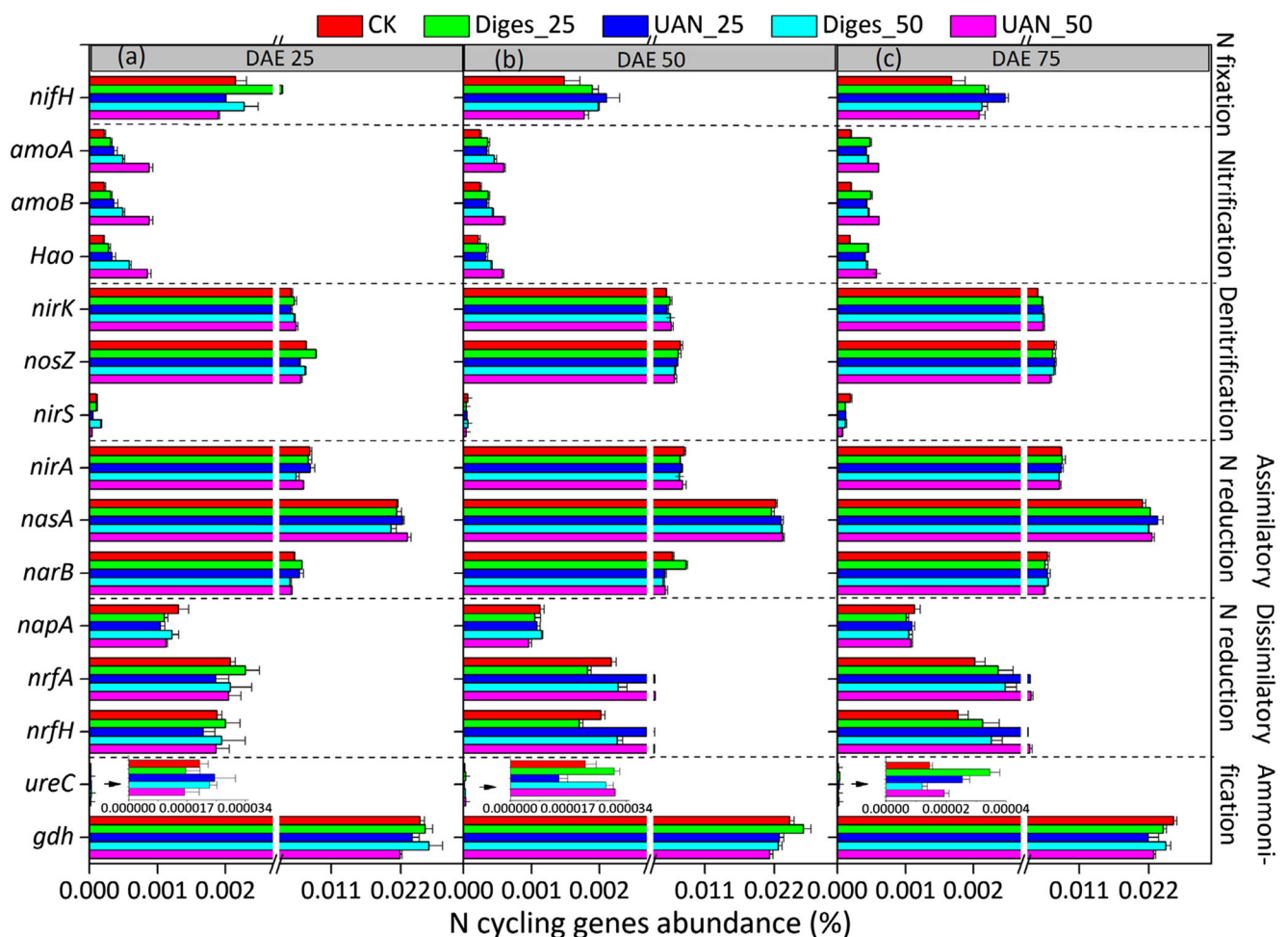


Fig. 5 Rhizosphere PICRUSt N cycling gene abundance of annual ryegrass assessed at 25, 50, and 75 days after emergence (DAE). See Fig. 1 for treatment descriptions. Error bars are the standard error of the mean

The diversity of predicted N cycling genes within the rhizosphere increased with both digestate and liquid urea ammonium nitrate fertilization treatments at DAE 75 (Fig. S3c). The digestate treatments decreased the diversity of C cycling genes (Fig. S3d, e and f). To visualize these effects, a NMDS plot was used to display overall distribution of predicted N reactions in association with different fertilizer and addition rates. Community analysis by PERMANOVA showed that community composition was influenced mostly by fertilizer ($P < 0.001$), but also by application rate(s) at DAE 25 and 50 (both $P < 0.01$) with distinct clustering (Fig. S2; Table S1). Communities assessed by C cycling processes showed that the fertilizer had a significant influence, but fertilizer rate had less influence (Fig. S1; Table S1). Furthermore, community analysis by PERMANOVA showed that N and C putative functional genes were significantly influenced by fertilizer ($P < 0.001$), harvest time ($P < 0.001$), and their interaction ($P < 0.001$) (Fig. S4a and b).

Structural equation modeling identifies key linkages among plant, soil, and microbial variables

The integrated responses of the overall soil-microbe system were investigated using structural equation modeling (SEM), which can reveal relationships of soil, microbial, and plants following digestate and liquid urea ammonium nitrate application and rates. The final models of digestate and liquid urea ammonium nitrate demonstrated a good fit to the data ($\chi^2 = 4.08$, $P = 0.665$ and $\chi^2 = 7.85$, $P = 0.449$, respectively). The models were significant at the 0.001 level with $R^2 = 0.19$ – 0.97 . Overall, increasing the digestate rate significantly increased C cycling gene diversity and significantly decreased N cycling gene diversity and bacterial community composition due to increased NO_3^- -N. Digestate addition directly affected the pH initially and then indirectly affected C cycling gene community composition by decreasing the soil pH. In addition, the AM fungal colonization was significantly correlated with the community composition of bacterial and ryegrass biomass (Fig. 6a). Furthermore, the ryegrass biomass was significantly affected by digestate addition, bacterial community composition, and C cycling gene diversity. Although bacterial diversity did not have significant direct effects on ryegrass biomass, it could indirectly affect biomass by affecting the bacterial community composition. Moreover, the content of NO_3^- -N, exchangeable NH_4^+ -N in soil, and soil pH directly affected the ryegrass biomass (Fig. 6a). In general, the liquid urea ammonium nitrate addition rate significantly affected bacterial N and C cycling gene diversity and community composition with increased soil NO_3^- -N, exchangeable NH_4^+ -N, and decreased soil pH. The colonization of roots by AM fungi was significantly correlated with the composition of the bacterial communities and the C and N cycling genes (Fig. 6b). Biomass was significantly directly

affected by liquid urea ammonium nitrate addition, AM fungal colonization, NO_3^- -N, exchangeable NH_4^+ -N, and pH (Fig. 6b).

Discussion

Effects on physicochemical and biological soil properties

Digestate from anaerobic digestion of food wastes has potential use as an organic amendment due to its high content of organic matter and significant amount of exchangeable NH_4^+ (Buhlmann et al. 2019). The changes in inorganic-N (NO_3^- -N and exchangeable NH_4^+ -N) in the soil after the application of digestate and liquid urea ammonium nitrate suggest rapid nitrification of the exchangeable NH_4^+ -N added in the digestate and in the liquid urea ammonium nitrate applied to the soil. Digestate and liquid urea ammonium nitrate contain a high proportion of exchangeable NH_4^+ -N, which can be nitrified quickly in soil, and a relatively low quantity of organic forms (Albuquerque et al. 2012b). The initial high NO_3^- -N concentration in the soil decreased in the successive sampling period, so there was no accumulation of NO_3^- -N in the soil at the end of the experiment. Nitrate can be taken up directly by plants and incorporated into tissues, but it also has a high potential for entering groundwater through leaching or entering the atmosphere through denitrification (García-Sánchez et al. 2015; Di and Cameron 2016). In addition, a previous study has shown that both exchangeable NH_4^+ and NO_3^- can be taken up by AM fungi which can transport N to their host plants (Govindarajulu et al. 2005). AM fungal hyphae are at least three orders of magnitude thinner than roots and can extend more than 10 cm beyond the root surface (Cavagnaro et al. 2005), allowing them to take up nutrients quickly and extensively. AM fungal hyphae may also be able to access N in the less mobile exchangeable NH_4^+ form, acquiring this form of N before conversion to NO_3^- (Hodge and Storer 2015). In this experiment, digestate application increased AM fungal hyphal length and reduced the NO_3^- -N content when compared with liquid urea ammonium nitrate.

Variations in either soil pH or electrical conductivity might affect nutrient availability and uptake, as well as the biomass, activity, and composition of the soil microbial community (Lauber et al. 2009). Indeed, soil pH decreased with the increase in amendment rate throughout the experimental period. Soil organic matter has been recognized to be crucial for improving soil quality and regulating many soil functions (García-Sánchez et al. 2015). Here, we report that the addition of digestate to soil provided easily available organic matter, mostly degradable in the short term, which did not contribute to the increase in the soil total C content (García-Sánchez et al.

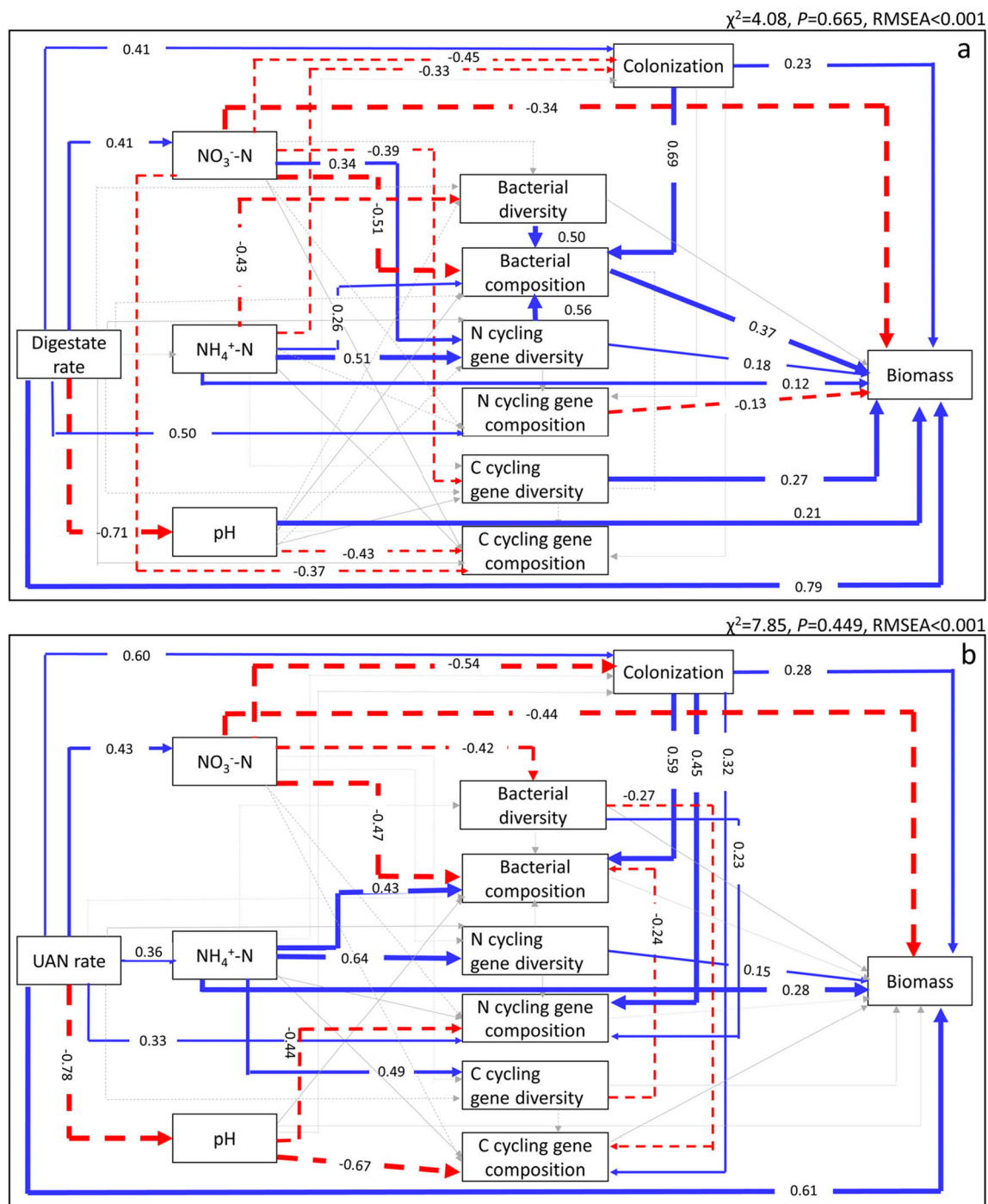


Fig. 6 The effects of soil properties, AMF and the bacterial diversity, richness and community composition on the annual ryegrass biomass estimated using structural equation modeling under digestate (**a**) and UAN (**b**) application. Blue lines indicate positive effects, while red

lines indicate negative effects. The width of arrows indicates the strength of significant standardized path coefficients ($P < 0.05$). Solid and dashed lines indicate positive and negative pathways, respectively. AMF: arbuscular mycorrhizal fungi; UAN: liquid urea ammonium nitrate

2015). Conversely, the content of dissolved organic C in soil treated with digestate increased significantly with harvest time. These findings suggest that an enhancement in dissolved organic C could originate from the release of organic substances during the decomposition of the organic matter within the digestate. This is consistent with

a previous study of García-Sánchez et al. (2015) which showed that the application of digestate led to an increase in dissolved organic C. This was probably because digestate application elevated available organic matter which was easily degraded and transformed by soil microbial activity.

Effects of digestate and liquid urea ammonium nitrate on plant growth, AM fungal colonization, and hyphal density

Plant growth showed significant improvements with application of both fertilizer types and rates in this soil which was N deficient for growth of ryegrass (see Methods). Walsh et al. (2012b) also reported that grasses to which digestate was applied had similar or better yields than those receiving inorganic N fertilizers, but this effect would depend on the original soil N. Digestate application, relative to inorganic fertilizer, may also increase soil organic matter and hence improve nutrient retention and soil quality (Albuquerque et al. 2012a).

Our observed reduction of AM fungal colonization with liquid urea ammonium nitrate addition supports previous studies where N-induced declines in AM fungal colonization (e.g. Gryndler et al. 2006; Jiang et al. 2018). This indicates that N enrichment of systems that are not P-limited reduces plant C allocation to mycorrhizal fungi (Johnson 2010). However, our amendment treatments did not reduce the abundance of AM fungal extraradical hyphae in the soil. The previous study showed that N addition reduced the arbuscular and vesicular colonization, but did not reduce the abundance of AM fungal extraradical hyphae (Jiang et al. 2018). Furthermore, our finding of increased development of AM fungal hyphae in soil following digestate application is in agreement with an earlier pot study (Gryndler et al. 2006). Such increased development of AM fungi in soil amended with organic matter could be related to a general increase in soil biological activity, where AM fungi may benefit from the release of other nutrients and growth-stimulating substances (Gryndler et al. 2006). However, in our case, the hyphal density of AM fungi was significantly decreased with the increase in liquid urea ammonium nitrate application rate. Similarly, Ngwene et al. (2013) reported that AM fungal hyphae density decreased with the supply of exchangeable NH_4^+ -N. Therefore, AM fungi could obtain less C allocation from the host and subsequently show lower hyphal density (Zheng et al. 2014).

Effect of digestate and liquid urea ammonium nitrate on rhizosphere bacteria and predicted functional gene

Changes in soil bacterial community composition associated with changes in quantity and quality of soil organic matter are likely to influence soil C storage and N cycling processes (Cusack et al. 2011; Zhang et al. 2019). Previous studies have observed shifts in soil bacterial composition following N (Zeng et al. 2016) or digestate application (Gielnik et al. 2019) to soil. Changes in community composition appeared quickly, being detected by DAE 25. However, rhizosphere microbiome changes were affected by the type of fertilizer (liquid urea ammonium nitrate or digestate) regardless of

application rate. There was lower bacterial community richness and diversity in the rhizosphere following the addition of liquid urea ammonium nitrate, which is consistent with previous demonstration of a decline in microbial diversity following N enrichment (Zeng et al. 2016). However, in our study, digestate application did not significantly decrease soil bacterial diversity and richness.

Digestate application increased the input of organic C and N in soil which have been shown to be involved in enhancement of diversity and richness of bacteria (García-Sánchez et al. 2015). Soil pH and exchangeable NH_4^+ -N availability are critical in determining bacterial community composition and diversity (Zeng et al. 2016). Lower soil pH can result in changes in nutrient availability (Stark et al. 2012), which may indirectly affect soil microbial community composition. Using a structural equation model (SEM), we found that the correlation of fertilization indirectly affected rhizosphere bacterial diversity and community by increasing soil NO_3^- -N. However, other factors may also contribute to soil microbial community composition changes in response to nutrient enrichment. Qin et al. (2016) found that AM fungal hyphae altered bacterial community composition. Our model showed that digestate and liquid urea ammonium nitrate addition significantly changed AM fungal colonization and hyphal growth which in return significantly affected bacterial composition.

DNA sequencing of the rhizosphere bacterial community indicated consistent general phylum-level responses associated with digestate and liquid urea ammonium nitrate addition in the soil. *Acidobacteria* and *Planctomycetes* generally decreased in abundance, while *Cyanobacteria*, *Firmicutes*, and *Actinobacteria* increased in abundance following liquid urea ammonium nitrate addition. Shifts in bacterial composition following N manipulation were previously explained by the copiotrophic hypothesis, in which copiotrophic groups (e.g., *Firmicutes* and *Actinobacteria*) that have fast growth rates are more likely to increase in nutrient-rich conditions, while oligotrophic groups (e.g., *Acidobacteria* and *Planctomycetes*) that have slower growth rate would likely decline (Fierer et al. 2007; Zeng et al. 2016). This shift is consistent with the microbial N mining hypothesis, which suggests that soil microbes reduce decomposition of recalcitrant C in response to lowered N requirement and lead to a shift towards labile C decomposition under N enrichment condition (Craine et al. 2007). In addition, digestate addition also significantly increased the relative abundance of *Firmicutes*. However, some oligotrophic organisms such as the *Cyanobacteria* did become more dominant following liquid urea ammonium nitrate addition. This indicated that phylum or class-level responses were mainly determined by changes at a lower taxonomic level, as not all bacterial taxa belonging to the same group shifted in a similar manner (Zeng et al. 2016). Also, bacterial rhizosphere responses were frequently inconsistent, and the response was

affected by both the amount of liquid urea ammonium nitrate and digestate added and the duration of the treatment (Janssens et al. 2010). For example, *Bacteroidetes* was significantly higher in relative abundance with digestate than with liquid urea ammonium nitrate (50 kg N ha⁻¹). Digestate amendment increased the relative abundance of *Bacteroidetes* at DAE 25, while there was no significant difference at DAE 75. The greater dominance of *Bacteroidetes* in the digestate treatments may, in part, reflect increased organic C availability (Eilers et al. 2012). Furthermore, the relative abundance of the dominant bacterial phyla was altered shortly after addition of digestate and liquid urea ammonium nitrate treatments, but these changes disappeared with time in the digestate treatment. This is consistent with a previous report that soil amendment with digestate did not have a major impact on soil microbial properties (Podmirseg et al. 2019). Thus, the autochthonous microbiota that prevails in the soil could be outcompeting and partially inhibiting the proliferation of allochthonous microorganisms. However, the long-term effect of digestate management to agricultural soil needs to be further studied.

Three genera more responsive to liquid urea ammonium nitrate addition were *Bacillus*, *Rhodococcus*, and *Streptomyces*. *Bacillus* and *Rhodococcus* have been shown to improve nutrient uptake, thus enhancing plant growth (Babalola 2010; Backer et al. 2018), which may have contributed to the higher biomass in the liquid urea ammonium nitrate (50 kg N ha⁻¹) treatment. The genus *Streptomyces* includes nitrogenase which can play a role in nitrogen fixation (Dahal et al. 2017). In addition, the genus *Rhodoplanes* may represent important group of free-living nitrogen-fixing bacteria (Buckley et al. 2007). The relative abundance of *Rhodoplanes* significantly decreased with liquid urea ammonium nitrate input when compared with digestate application. Thus, the genus *Rhodoplanes* may be suppressed by liquid urea ammonium nitrate addition. However, further studies are needed to demonstrate its potential function for plant growth promotion, which may be useful in agriculture.

The 16S rRNA gene profiling information from PICRUST (Langille et al. 2013) was used to predict the abundance of C and N functional genes under different fertilizer applications. Fertilization (digestate, liquid urea ammonium nitrate) treatments influenced most predicted rhizosphere genes involved in C degradation and N cycling. Previous studies reported that the function of microbial communities was affected by different fertilization practices (He et al. 2007), and this may accelerate soil C and N turnover. In these studies, the *nifH* gene plays a key role in the biological conversion of atmospheric N to exchangeable NH₄⁺ or fixed N₂ (Fani et al. 2000). Consistent with the enhanced abundance of *nifH* following N fertilization observed by Zhang et al. (2019), we found an increase in *nifH* with digestate and liquid urea ammonium nitrate addition at DAE 50 and 75. N-fixing bacilli are often isolated from rhizosphere soil (Achouak et al. 1999) and

Bacillus was a most abundant genus whenever digestate and liquid urea ammonium nitrate were applied to soil. In addition, there was a decrease in *nifH* with increasing levels of liquid urea ammonium nitrate addition. However, in our study, stability in the predicted rhizosphere's *nifH* gene abundance was observed after addition of digestate fertilizer. Fertilizer affects the N₂ fixing bacteria present in plant rhizospheres in different ways, depending not only on the different fertilizer, but also possibly on the soil type (Li et al. 2014b), nutrient availability (Waldrop and Zak 2006), and soil acidification (Ning et al. 2015).

Nitrification is an important process in N metabolism (Su et al. 2015). The main genes involved include *hao*, *amoA*, and *amoB*. The abundances of *amo* and *Hao* increased with digestate and liquid urea ammonium nitrate addition and this was consistent with a previous study (Zhang et al. 2019) where *amo*- responded positively to exchangeable NH₄⁺-N and NO₃⁻-N enhancement. Although digestate application increased the abundance of *amoAB* and *Hao*, their abundance in the rhizosphere was significantly decreased when compared with liquid urea ammonium nitrate addition. Thus, addition of digestate may reduce soil acidification. However, appropriate digestate management and processing practices are needed to avoid potential acidification and eutrophication impacts due to increased nutrient leaching (Albuquerque et al. 2012b) although this is dependent on the local soil quality and meteorological conditions as well as digestate characteristics (Evangelisti et al. 2014).

Liquid urea ammonium nitrate application increased the abundance most of the genes involved in C degradation, suggesting that fertilization can accelerate soil C turnover in this sandy soil. Addition of liquid urea ammonium nitrate and digestate both enhanced the abundance of genes related to soil oxidoreductase (catalase). This may be the consequence of stimulation of both microbial growth and activity via improved nutrient availability as well as changes in microbial community composition induced by fertilizer addition (Ge et al. 2009; Ai et al. 2012). However, the predicted gene abundance in the rhizosphere responsible for chitin and more labile C (arabinofuranosidase, xylanase, beta-galactosidase) degradation was decreased by liquid urea ammonium nitrate application when compared with digestate. This is likely to reduce the content of labile degradable C input in soil (Yang et al. 2020). The digestate had a lesser effect on C degradation genes which is consistent with a previous study (Möller 2015) and may be important in maintaining C stability in soil. However, we only determined the presence of functional genes and not their expression, and we did not quantify enzyme activities. The presence of microbial groups which can carry out a given function in soil cannot be used as proxy of potential microbial functions of soil (Nannipieri et al. 2020). Thus, further research is needed to consider the linkage between microbial functional genes and enzyme activities of soils.

Conclusions

This study demonstrated that digestate added at an equivalent N content to urea (liquid urea ammonium nitrate) was equally effective as a fertilizer for annual ryegrass growth. However, digestate increased the development of AM fungal hyphae density, but the amount of liquid urea ammonium nitrate addition significantly reduced AM fungal hyphae density. The amount of fertilizer application had little influence on the bacterial phylogenetic diversity and composition, and over time, different forms of N fertilizer and application rates can significantly shift rhizosphere bacterial community composition. The abundance of most of the functional genes involved in C and N cycling were significantly stimulated after digestate and liquid urea ammonium nitrate amendment. Although use of digestate had a lesser impact on soil microflora diversity and potential function than the liquid urea ammonium nitrate fertilizer, the community composition and N and C putative functional genes changed with time. Resistance and resilience of the main microbial groups and their activity need to be evaluated. Quantification of the targeted expressed genes would contribute to understanding the function of microbial communities (Nannipieri et al. 2019).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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