



Labile carbon inputs support the recovery of bacterial communities, but not fungal communities, from a simulated bovine urine event

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Received: 26 August 2022 / Revised: 16 February 2023 / Accepted: 20 February 2023 / Published online: 28 February 2023
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Abstract

Inputs of carbon to soil may be used to stimulate microbial growth and immobilize excess nitrogen from sources such as livestock urine. However, the growth responses of microbial taxa to carbon inputs under conditions of excess soil nitrogen remain poorly understood. Using DNA metabarcoding and a field-based soil lysimeter experiment, we characterised the temporal responses (up to 112 days) of bacterial and fungal communities to a simulated bovine urine event plus inputs of labile carbon (sucrose) at two concentrations. Fungal communities were impacted more strongly than bacterial communities by carbon inputs following the simulated urine event, with more variable responses among taxa. Chytridiomycota and Glomeromycota richness were most negatively affected, and Tremellomycetes richness most positively affected, by carbon inputs. A minority of fungal ASVs had greatly increased proportional abundances in response to carbon, while fungal trophic composition became highly dominated by saprotrophs by the experiment end. Bacterial taxa showed consistent trends of declining (to about 14 days) and recovering (to 112 days) richness in response to urine and carbon inputs, but carbon-related evenness and proportional abundance trends varied between taxa. Proportional abundances of Actinobacteria, Bacteroidetes, Betaproteobacteria, and Gammaproteobacteria increased in response to carbon, whereas proportional abundances of Acidobacteria, candidate division WPS-1, Planctomycetes, Deltaproteobacteria, and Verrucomicrobia decreased. These results show that labile carbon inputs to limit nitrate leaching support the recovery of bacterial communities to bovine urine events but may have long-term impacts on fungal community composition and function, with potential consequences for soil food webs, carbon sequestration, and agricultural productivity.

Keywords Bacteria · Fungi · Soil biodiversity · Urine · Carbon · Nitrogen

Introduction

Nitrogen-containing fertilizers are widely used to increase the productive capacity of agricultural ecosystems. However, agricultural nitrogen inputs often have effects beyond the immediate locations where they are applied. Nitrogen is subject to a myriad of biogeochemical transformations, often transitioning through chemical forms that are highly mobile and transported through soil, into waterways, or the

atmosphere. Thus, these inputs have meant that the world's ecosystems, in general, are undergoing nitrogen enrichment, perturbing the global nitrogen cycle (Jefferies and Maron 1997; Stevens 2019). Consequent issues include nitrate/nitrite enrichment of water (Ward et al. 2018), eutrophication (McDowell et al. 2009; Dodds and Smith 2016), greenhouse gas emissions (Ciais et al. 2013), and stratospheric ozone depletion (Ravishankara et al. 2009). Indeed, agriculture is the main source of the powerful greenhouse gas N₂O (Reay et al. 2012). Overall, these effects of nitrogen enrichment are well recognised but addressing them remains a major challenge.

A critical pathway for nitrogen deposition in agricultural ecosystems, alongside mineral fertiliser use, is via inputs of animal waste, primarily urine. This pathway is important as deposition by livestock creates spatially and temporally heterogeneous patches of soil nitrogen (Auerswald et al. 2010) that vastly exceed the capacity

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for plants to capture from the soil and/or utilize (Haynes and Williams 1993). This mismatch between nitrogen supply and demand results in localized patches of excessive soil nitrogen concentrations (Vitousek et al. 2009; Sousana and Lemaire 2014), which can be converted to environmentally damaging forms. The effective management of such nitrogen deposits is therefore key to preventing adverse effects of agriculture (Zhang et al. 2015).

Livestock urine patches are also characterised by localised increases in electrical conductivity, pH, and osmotic potential (Haynes and Williams 1992), in addition to high urine concentrations. These changes in soil solution chemistry have impacts on soil microbial communities that depend on urine composition and soil conditions (Petersen et al. 2004; Orwin et al. 2010; Bertram et al. 2012). Urea triggers a sequence of microbial community succession, which is partially mediated by competition (Ganasamurthy et al. 2021) and might differently affect prokaryotes (bacteria and archaea) and slower-growing fungi. Higher ratios of fungi to bacteria in soil are typically associated with increased carbon sequestration (Bailey et al. 2002; Malik et al. 2016), and may be indicative of low-input self-regulating communities (Bardgett and McAlister 1999). High fungal biomass may also be an indicator of increased retention of nitrogen in soils (de Vries et al. 2011), while mounting evidence suggests soil fungi are important contributors to nitrous oxide emissions (Phillips et al. 2016; Aldossari and Ishii 2021; Ganasamurthy et al. 2021). Inputs of urine to soil also affect microbial carbon cycling (Lambie et al. 2013). In locations of one-off or repeated urine deposits, relative shifts of fungal and prokaryotic communities may therefore underpin changes to important soil functions.

One approach to managing nitrogen levels in soil is via carbon-stimulated microbial growth. Levels of carbon, nitrogen, and phosphorus in soil microbial biomass are strongly correlated, with a global average C:N ratio of 7.6 (Xu et al. 2013). Soil microbes tend to be carbon limited (Soong et al. 2020), and growth of soil microbial biomass requires some 5–10 times more carbon than nitrogen. Thus, carbon inputs to soil stimulate microbial growth and the immobilisation of soil nitrogen, reducing the concentration of easily leached forms of nitrogen. Carbon inputs have therefore been used to reduce nitrogen leaching losses from intensive horticulture (Chaves et al. 2008) and pastoral agriculture (Ledgard et al. 2007; Shepherd et al. 2010; Talbot et al. 2019). However, given the numerous process feedbacks between aboveground livestock, plants, soil resources and soil organisms, the effects of carbon inputs in addition to high nitrogen loads on soil communities in grazed pastoral systems are unclear. Until these effects are better understood, we cannot predict whether increasing the amount of readily utilisable carbon will mitigate nitrogen leaching from urine deposition in the long-term, nor assess what unintended consequences this approach might have.

Microorganisms have varied physiological attributes and nutritional requirements (Van Gestel et al. 1993; Garnier et al. 2003). Therefore, inputs of carbon can be reflected in the selection of communities with differing fundamental growth rates and demand for nutrients such as nitrogen and phosphorus. This provides a basis for classifying microorganisms based on growth rates, with terminology such as ‘autochthonous/zymogenous’ and ‘oligotrophic/copiotrophic’, indicating low/high nutrient requirements and growth rates, having been used widely (Langer et al. 2004). These categories are microbial equivalents of the *r/K* selection concept applied to animals and plants (Planka 1970; Fierer et al. 2007). More recently, models such as ‘YAS’ and have been proposed for soil microbes, incorporating both resource availability/limitation and ecosystem stress (Malik et al. 2020).

Talbot et al. (2019) demonstrated that inputs of labile carbon (as sucrose) at two different levels substantially reduced leaching of nitrogen from bovine urine patches in pastoral soil planted with mixed ryegrass (*Lolium perenne*) and clover (*Trifolium repens*) over the timespan of 4–6 months following a one-off simulated urination event, in proportion to the level of labile carbon added. In this study, we follow on from Talbot et al. (2019) by investigating the effects of these labile carbon inputs on bacterial and fungal communities in the same pastoral soils after the same simulated bovine urination event. We investigated the responses of soil communities in urine patches to two levels of labile carbon inputs, and whether they recover to the same biodiversity and functional composition over time. Furthermore, given their differing lifespan and turnover rates (Rousk and Bååth 2011), do bacteria and fungi show the same temporal responses to urine plus carbon inputs, and if not, how might this alter soil functions? We used a DNA metabarcoding approach to analyse temporal biodiversity, composition, and functional trends for bacterial and fungal communities for up to 112 days following urine deposition. We tested the following null hypotheses: (1) That inputs of labile carbon support the recovery of both bacterial and fungal communities in soil from the impacts of a bovine urine event; (2) that bacterial and fungal temporal responses do not differ between two different levels of labile carbon input; (3) and that temporal responses to these inputs do not differ between different taxa within bacteria and fungi.

Methods

Experiment design and sampling

The experiment was established at Lincoln University’s Ashley Dene Research & Development Station, located on

the East coast of New Zealand's South Island at latitude 43°38.85' S longitude 172°20.76' E, 17 m above sea level. This study forms part of a wider soil lysimeter experiment described in full by Talbot et al. (2019). In June 2017 (winter), rings were driven into the soil to extract 15 soil blocks, each 500 mm in diameter and 200 mm deep, from mixed ryegrass/clover (*Lolium perenne/Trifolium repens*) pasture and placed upon a bed of sand within a trench. All spaces around the blocks were filled with topsoil. The soil blocks were manipulated in a manner consistent with typical dairy farming practices throughout the experiment (Table 1), as follows. Fertiliser was applied on 30 June 2017; this comprised 11 g superphosphate per block (equivalent to 0.555 t ha⁻¹) and 20 g hydrated lime per block (equivalent to 1 t ha⁻¹) with 4 l water per block. This adjusted Olsen P and pH levels to those typical of dairy farm values (Sparling and Schipper 2004). A further application of hydrated lime (20 g per block, equivalent to 1 t ha⁻¹, with 4 l water per block) was made on 18 September 2017. Irrigation was applied from 4 October 2017 until the end of the experiment at a rate of 5–10 mm water per application.

Each of the soil blocks was randomly allocated to one of three different experimental treatments (five blocks per treatment). The treatments consisted of single applications of (a) urine (only) at 700 kg N ha⁻¹ (N-only); (b) urine at 700 kg N ha⁻¹ plus labile carbon (sucrose) at 5.1 t C ha⁻¹ (N + C1); and (c) urine at 700 kg N ha⁻¹ plus carbon at 10.1 t C ha⁻¹ (N + C2). The urine was collected from lactating Friesian/Jersey-cross cattle fed upon mixed ryegrass/clover, adjusted to 6.9 g N l⁻¹ by addition of 703.1 g urea to 125 l urine, of which 2 l was applied to each of the soil blocks. The carbon was added in the form of sucrose dissolved in water at rates of 221.7 g (C1) and 443.4 g (C2) sucrose l⁻¹, of which 1 l were applied per N + C1 and N + C2 soil block, respectively.

Table 1 Timeline of experimental manipulations and soil sample collections

Season	Date	Days	Experimental treatments	Soil sample
Winter	Jun. 30	-4	Applications of superphosphate and hydrated lime, simulated grazing	none
	Jul. 02	-2	Sucrose application	none
	Jul. 03	-1	Simulated trampling	none
	Jul. 04	0	Urine application	T0
	Jul. 05	1	none	T1
	Jul. 11	7	none	T2
	Jul. 18	14	none	T3
	Aug. 01	28	none	T4
	Aug. 29	56	none	T5
	Spring	Sep. 19	77	Application of hydrated lime
Oct. 05		93	Irrigation began	none
Oct. 24		112	End of experiment	T6

Water (1 l) was added to the N-only soil blocks at the same time as the carbon treatments, to ensure consistent moisture inputs for all three treatments.

At the start of the experiment, simulated grazing and trampling effects were applied, along with the carbon and urine applications, on successive days (Table 1). The day of the first soil sampling (4 July 2017) was designated T0. Simulated grazing (cropping of vegetation with electric shears) was applied to all blocks four days before T0 (the same day as fertiliser application). The carbon was applied to N + C1 and N + C2 blocks two days before T0. Simulated trampling (six stomps per block by a simulated cow hoof) was applied to all blocks one day before T0. The first soil samples for DNA metabarcoding were collected at T0, immediately after which the urine was applied to all blocks. Further soil samples were collected after 1, 7, 14, 28, 56 and 112 days (24 October 2017), designated T1–T6 (105 samples in total). The soil samples were collected using a 75 mm diameter soil corer to a depth of 100 mm. The holes caused by soil sampling were each subsequently backfilled with a soil-sand mix and marked to avoid future sampling at the same point.

DNA extraction, PCR, and sequencing

DNA was extracted from triplicate subsamples (0.25 g) from each of the 105 samples, along with DNA extraction blanks (containing only sterile water), urine samples, and sand (used to back-fill sample holes), using a Macherey–Nagel Nucleospin Soil DNA extraction kit (MACHEREY–NAGEL GmbH & Co. KG, Düren, Germany). Aliquots of the triplicate DNA extracts were pooled together for each sample and quantified using the Quant-iT PicoGreen dsDNA assay (Invitrogen, Eugene, OR, USA) and the LabChip Genomic DNA assay (PerkinElmer, Hopkinton, MA, USA).

Two barcode genes were each amplified in duplicate PCRs from each sample: prokaryote 16S using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACGVGGGTWTCTAAT-3') (Caporaso et al. 2011); fungal ITS using primers FITS7 (5'-GTGARTCATCGAATCTTTG-3') (Ihrmark et al. 2012) and ITS4 (5'-TCC TCCGCTTATTGATATGC-3') (White et al. 1990). The primers were respectively modified at their 5' ends with the linker sequences 5'-TCGTCGGCAGCGTC-3' and 5'-GTC TCGTGGGCTCGG-3'. PCRs were carried out in 25 µl volumes, containing 500 nM of forward and reverse primers, 2 µg rabbit serum albumin, 0.5 U KAPA Plant 3G enzyme and 12.5 µl 2 × KAPA Plant 3G Buffer, (Kapa Biosystems, Wilmington, MA, USA), with 2 µl DNA template. The PCR amplification protocol for bacterial 16S was 95 °C for three minutes; 25 cycles of 95 °C for 20 s, 55 °C for 15 s, and 72 °C for 30 s; and one minute at 72 °C. For fungal ITS: 95 °C for three minutes; 30 cycles of 95 °C for 20 s, 52 °C for 15 s, and 72 °C for 30 s; and one minute at 72 °C.

Illumina sequencing adapters and sample-specific barcodes were added to the amplicons in a second PCR, carried out in 25 μ l volumes containing the containing 2 μ g rabbit serum albumin and 12.5 μ l 2 \times KAPA HiFi ReadyMix Buffer, (Kapa Biosystems, Wilmington, MA, USA), with 500 nM Illumina-tagged sequencing adapters instead of target primers, and 1 μ l of the first PCR amplicons as template. The PCR amplification protocol was 95 °C for three minutes; five cycles of 98 °C for 15 s, 65 °C for 15 s, and 72 °C for 15 s; and one minute at 72 °C. The resulting amplicons were purified and size-selected using SPRIselect magnetic beads (Beckman Coulter, Brea, CA, USA), to remove primer dimers and high molecular weight DNA, quantified, pooled together. The pooled bacterial 16S and fungal ITS amplicons were each sequenced in a 2 \times 250 bp MiSeq run.

Bioinformatic processing

Demultiplexed forward and reverse DNA sequences were merged and labelled according to sample using VSEARCH (Rognes et al. 2016). Sequencing adapters and PCR primers were trimmed from the merged sequences using cutadapt (Martin 2011). Using VSEARCH again, the trimmed sequences were filtered to remove any with > 1 maximum expected errors or lengths < 150 bp or > 450 bp. The sequences were then denoised into absolute sequence variants (ASVs), and filtered for chimeras. ASV abundance was inferred by mapping the trimmed sequences back to the ASV sequences at a sequence identity threshold of 97%. The bacterial 16S and fungal ITS ASVs were assigned a taxonomic identity using the RDP naïve Bayesian classifier (Wang et al. 2007) with the default prokaryote database or the UNITE fungal ITS database (Nilsson et al. 2019) option, respectively. The metabolic pathway composition of bacterial communities in each treatment and at each time point was inferred using PICRUSt2 (Douglas et al. 2020). The trophic modes and growth forms of fungal ASVs in each treatment and at each time point were determined using the FungalTraits database (Pöhlme et al. 2020).

Biodiversity analyses

All data analyses were carried out in RStudio using R version 3.5.2, including the packages vegan (Oksanen et al. 2017) for biodiversity analyses, dplyr (Wickham et al. 2022) for data manipulations, and ggplot2 (Wickham 2009) for generating figures. DNA extraction blanks, negative and positive controls were examined for contamination. Tag jumping (Schnell et al. 2015) was accounted for by using a regression of contaminant abundances versus the maximum of total abundances in all other samples, after which the coefficient estimate for the 90th quantile regression was used

to subtract that many sequences from the abundances of all ASVs (Makiola et al. 2019).

Multivariate community composition was compared among times and treatments using non-metric MDS ordinations and PERMANOVA tests (Anderson 2017), based on Bray–Curtis dissimilarities between relative ASV abundances per sample.

To investigate the resilience of bacterial and fungal communities to nitrogen and carbon inputs, data from each set of five treatment/time replicates was pooled together, followed by the calculation of (a) the proportions of ASVs detected at T0 that were also detected at time points T1 through T6; (b) the proportions of ASVs detected at T0 that were not also detected at time points T1 through T6; and (c) the proportions of ASVs detected at T1 through T6 that were not also detected at T0 (relative to the number of ASVs at T0).

Changes in the richness, evenness, and proportional abundance of bacterial 16S and fungal ITS communities over time were determined for each of the experimental treatments, for overall communities, and for the main taxonomic phyla and classes within each dataset. To account for varying sequencing library depths among samples and time points, the ASV abundance tables for each dataset were randomly subsampled 99 times to a consistent sequencing depth (20,000 reads per sample for bacterial 16S, 1,500 reads for fungal ITS). ASV richness and evenness estimates were then determined for each sample (overall, or for taxonomic subsets) as the mean biodiversity of that sample across the 99 subsampled datasets (Makiola et al. 2019). To calculate proportional abundances, non-subsampled ASV abundances per sample were converted to proportions of the entire non-subsampled dataset. To analyse treatment effects on temporal changes in richness, evenness and proportional abundance, T0 values were considered a baseline and set to zero, and values for time points T1–T6 (1–112 days) were converted to relative values compared to those at T0.

Linear mixed models were used to test for polynomial temporal biodiversity trends, and for differing trends between treatments, using the R package lmerTest (Kuznetsova et al. 2017). Treatment (N-only, N + C1, and N + C2) was considered as a fixed factor, time (number of days) as a fixed continuous polynomial with degree of up to 3, and soil block as a random variable. Changes in ASV richness, evenness, and proportional abundance per taxonomic group were visualised as radar plots for (a) the time point when biodiversity metrics for most bacterial and fungal taxa were maximally different from T0 values (T3/14 days for bacteria, T4/28 days for fungi), and (b) the experiment end (T6/112 days).

To investigate bacterial community metabolic responses to urine and labile carbon inputs, changes in PICRUSt2-inferred bacterial metabolic pathway abundances over time were determined relative to pathway abundances at the start

of the experiment (T0) and visualised using heatmaps. To investigate fungal community function responses to urine and labile carbon inputs, changes in the relative abundances of fungal trophic modes and growth forms over time were determined for ASVs assigned to genera that matched those represented in the FungalTraits database (Pöhlme et al. 2020). Fungal trophic modes and growth modes representing less than 1% of the total abundance of these matched ASVs were pooled together as “Others”.

Results

Temporal effects of urine and carbon inputs on bacterial and fungal communities

Non-metric MDS ordinations showed limited evidence of changing bacterial community composition over time in

the N-only treatment, but increasing divergence of bacterial communities from their initial state with increasing carbon input levels until at least 28 days (T4) in the N + C treatments, after which time they converged back towards their initial states (Fig. 1a). Fungal communities become increasing divergent from their initial states with time and with increasing carbon input levels, to a greater extent than the bacterial communities (Fig. 1b). The largest carbon-driven shifts in bacterial and fungal community composition between consecutive time points evidently occurred between 1 and 7 days (T1-T2). PERMANOVA tests detected evidence for significantly differing bacterial and fungal community compositions between treatments ($F_2 = 2.953$ and 3.201 , $p \leq 0.001$) and across times ($F_1 = 4.058$ and 3.125 , $p \leq 0.001$; Online Resource 1). PERMANOVA tests between communities detected at the start (T0) and end (T6) of the experiment indicated that neither bacterial ($F_1 = 3.456$, $p \leq 0.001$) nor fungal

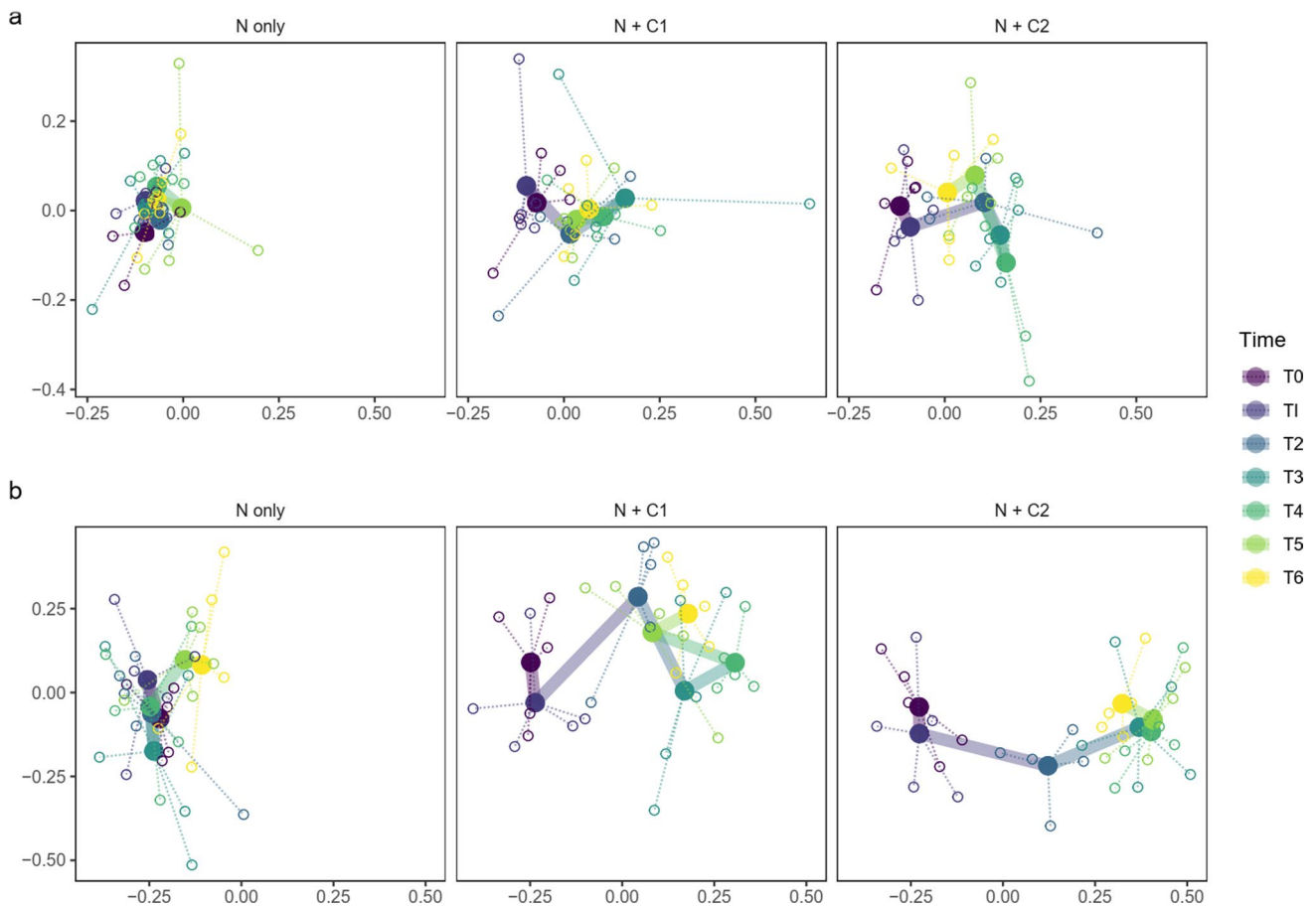


Fig. 1 Temporal changes in the community structure of (a) soil bacterial and (b) soil fungal communities, before (T0) and after (T1-T6) applications of bovine urine with N at a rate of 700 kg N ha^{-1} (N-only), or urine plus labile carbon (sucrose) at rates of 5.1 t C ha^{-1} (N+C1) and 10.1 t C ha^{-1} (N+C2), based on Bray–Curtis dissimilarities between relative ASV abundances per sample. T0 through T6

correspond to samples collected immediately before, and 1, 7, 14, 28, 56 and 112 days after application of urine. Carbon was added two days before T0. Open circles represent sets of five replicate samples, connected by dotted lines to their respective multivariate centroids, which are represented by filled circles. Centroids from consecutive time points are linked by a solid line

communities ($F_7 = 2.949$, $p \leq 0.001$) had returned to their initial composition after 112 days.

Over half of the bacterial ASVs detected in the first samples (T0) were also detected at all subsequent time points in each experimental treatment, except for 14 days (T3) in the N-only treatment (Fig. 2a). Smaller proportions of T0 bacterial ASVs were not detected at subsequent time points in the N+C treatments (25% to 41%) compared to the N-only treatment (31% to 53%). Conversely, larger proportions of bacterial ASVs were newly detected at each time point from 1 to 112 days (T1 to T6) in the N+C treatments (13% to 43%) compared to the same time points in the N-only treatment (6% to 28%).

The proportions of fungal ASVs changed to a much greater extent than the proportions of bacterial ASVs throughout each treatment (Fig. 2b). In the N-only treatment, less than half of the T0 fungal ASVs (29% to 39%) were also detected at subsequent time points, with up to 76% of T0 ASVs not detected subsequently, and up to 57% of ASVs newly detected at each time point after T0. Carbon inputs resulted in increased losses of fungal ASVs, with less than 6% of T0 fungal ASVs detected after 28 days (T4) in the N+C treatments. Furthermore, the proportions of fungal ASVs newly detected after 7 to 112 days (T2–T6) in the N+C treatments (14% to 35%) were greatly exceeded by T0 ASVs that were absent at these time points (82% to 97%).

Temporal biodiversity trends for Bacteria

The mean richness of overall bacterial communities in each treatment declined from the experiment start to 14 days (T3)

by similar amounts – up to 959 ASVs (23%) in the N+C2 treatment – before increasing again to 112 days (T6) (Online Resource 2, Fig. S1a). Evenness trends in the N+C treatments were similar to the richness trends, whereas evenness changed little in the N-only treatment (Online Resource 2, Fig. S1b). Significant negative cubic trends were detected for both richness and evenness ($F_{87} = -5.23$ to -4.69 , $p \leq 0.001$; Online Resource 1), plus a significant evenness (but not richness) difference between the N-only and N+C2 treatments ($F_{12} = -3.26$, $p = 0.007$). Mean proportional abundance of overall bacterial communities increased slightly in the N+C treatments but declined in the N-only treatment (Online Resource 2, Fig. S1c), but these trends were not statistically significant.

Biodiversity metrics for individual bacterial taxa most commonly reached maximal divergence from T0 values after 14 days (T3). Richness declines for all examined taxa were smaller in the N+C treatments than in the N-only treatment (Fig. 3 & Online Resource 2, Fig. S2a). For example, the mean richness of Planctomycetes declined by 41% (394 ASVs) after 14 days (T3) in the N-only treatment, compared to 13% (98 ASVs) and 20% (152 ASVs) at the same time point, in the N+C1 and N+C2 treatments, respectively. By the end of the experiment, mean richness was higher than initial values for seven (N+C1) or ten (N+C2) of 12 bacterial taxa, but only four taxa in the N-only treatment. Significant negative cubic richness trends were detected for all examined taxa except Bacteroidetes ($F_{87} = -2.40$ to -4.12 , $p \leq 0.001$; Online Resource 1). Only marginally significant differences between richness levels in N+C treatments

Fig. 2 Resilience of (a) bacterial and (b) fungal soil communities to applications of bovine urine with N at a rate of 700 kg N ha⁻¹ (N-only), or urine plus labile carbon (sucrose) at rates of 5.1 t C ha⁻¹ (N+C1) and 10.1 t C ha⁻¹ (N+C2). Legend categories represent the proportions of T1–T6 ASVs that were not also detected at T0 (new), the proportions of T0 ASVs that were also detected at T1–T6 (retained), and the proportions of T0 ASVs that were not also detected at T1–T6 (lost), relative to the number of ASVs at T0, based on pooled data from five replicate samples at each time point. T0 through T6 correspond to samples collected immediately before, and 1, 7, 14, 28, 56 and 112 days after application of urine. Carbon was added two days before T0

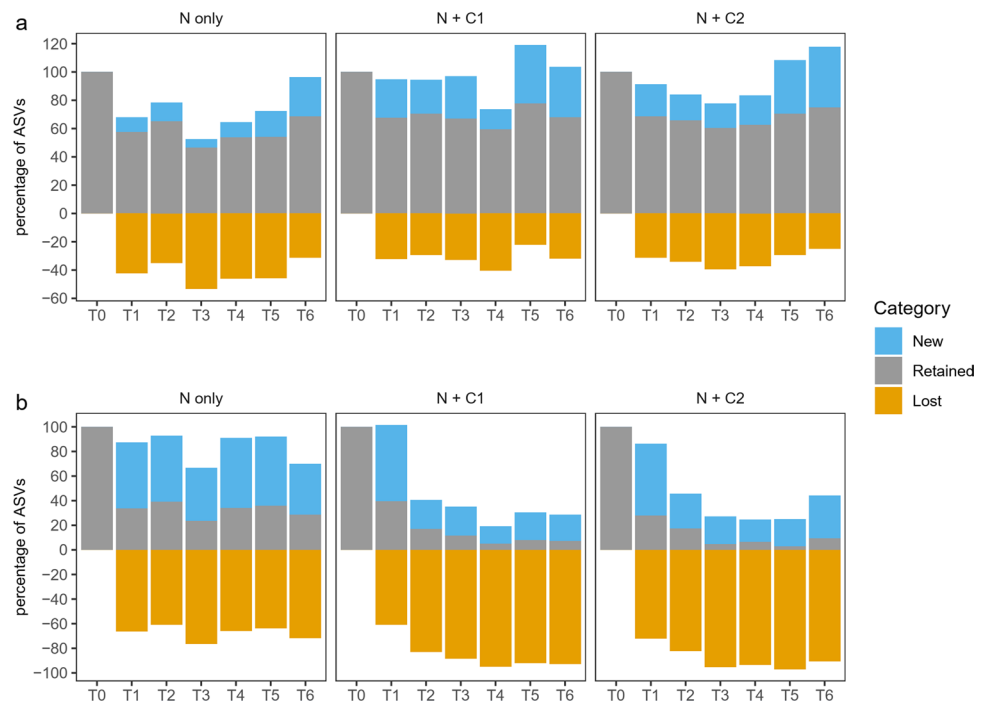
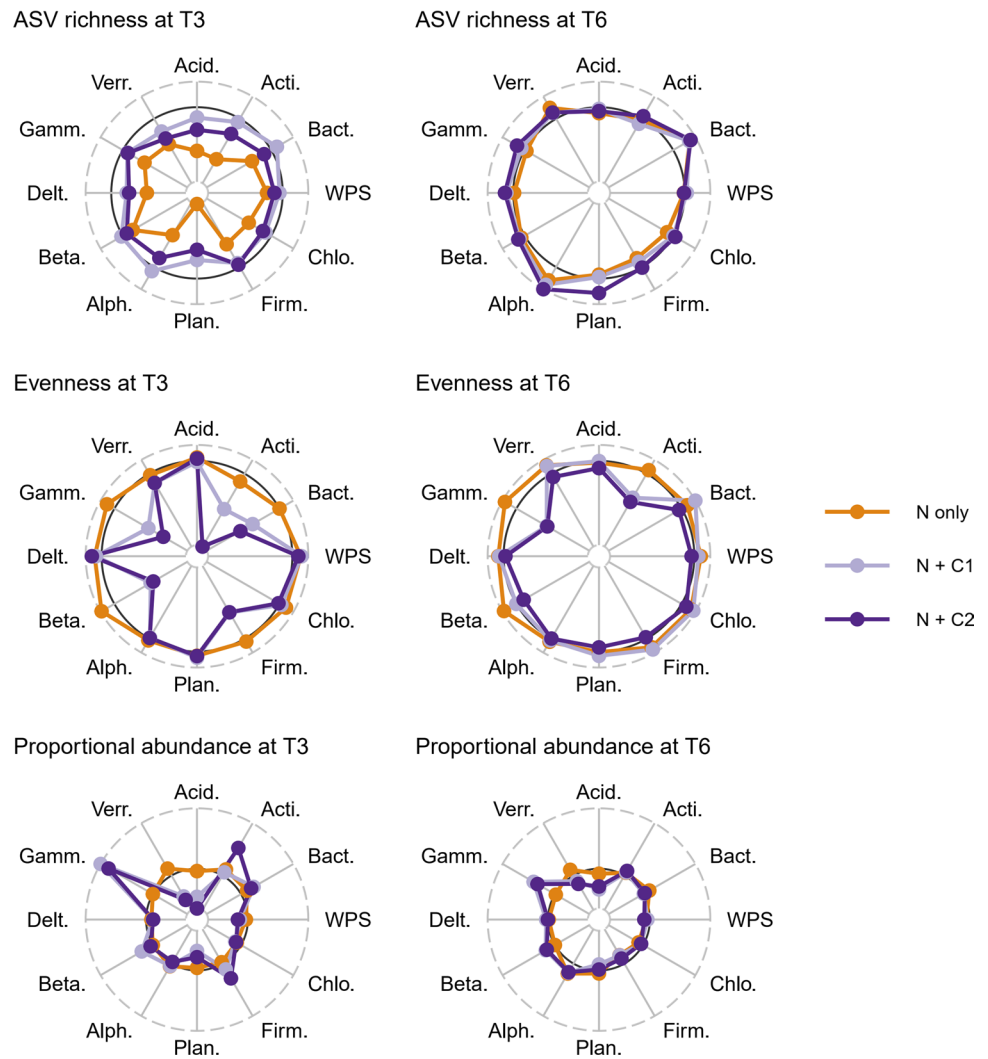


Fig. 3 Mean ASV richness, evenness, and proportional abundances of the twelve most common (non-proteobacterial) phyla and (proteobacterial) classes of bacteria, 14 days (T3) and 112 days (T6) following applications of bovine urine with N at a rate of 700 kg N ha⁻¹ (N-only), or urine plus labile carbon (sucrose) at rates of 5.1 t C ha⁻¹ (N + C1) and 10.1 t C ha⁻¹ (N + C2), relative to values at T0, immediately before urine input. Solid circles represent mean values at T0, while grey dashed circles represent minimum and maximum biodiversity values at either T3 or T6. Taxonomic label abbreviations are as follows: Acidobacteria; Actinobacteria; Bacteroidetes; candidate division WPS-1; Chloroflexi; Firmicutes; Planc-tomyces; Alphaproteobacteria; Betaproteobacteria; Deltaproteobacteria; Gammaproteobacteria; Verrucomicrobia



compared to N-only were detected, however, for Firmicutes in N + C2, Chloroflexi in N + C1, and Betaproteobacteria in N + C1 ($F_{12} = 1.98$ to 2.13 , $p = 0.71$ to 0.054).

Evenness and proportional abundance trends were more varied among bacterial taxa than richness trends (Fig. 3 & Online Resource 2, Fig. S2b & c). In the N + C treatments, the mean evenness of Actinobacteria, Bacteroidetes, Firmicutes, Betaproteobacteria, and Gammaproteobacteria all declined from the experiment start to 14 days (T3), and in contrast to their N-only trends, by 12% for Bacteroidetes and Firmicutes in N + C1 to 31% for Actinobacteria in N + C2. The evenness of each of these taxa except Actinobacteria and Gammaproteobacteria recovered to similar levels as initial values after 112 days (T6). These same taxa each had substantial increases in mean proportional abundance over time, with maximal increases of 26% for Firmicutes after 14 days (T3) in N + C1 to 304% for Gammaproteobacteria after 7 days in N + C2. The proportional abundances of all these taxa except Firmicutes were still higher than their initial values after 112 days (T6), by up to 103% for

Gammaproteobacteria in N + C1. Conversely, the mean proportional abundances of Acidobacteria, candidate division WPS-1, Planctomycetes, Deltaproteobacteria, and Verrucomicrobia in N + C treatments each declined over time (and relative to their N-only trends), by 24% for Acidobacteria after 14 days (T3) in N + C1, to 56% for Deltaproteobacteria after 28 days (T4) in N + C2, while evenness trends for these taxa showed little change. Significant negative cubic evenness trends were detected for Actinobacteria, Bacteroidetes, Firmicutes, Betaproteobacteria, and Gammaproteobacteria ($F_{12} = -2.66$ to -4.06 , $p = 0.009$ to <0.001 ; Online Resource 1), while significant positive cubic evenness trends were detected for Planctomycetes and Deltaproteobacteria ($F_{12} = 2.21$ and 2.09 , $p = 0.029$ and 0.040 , respectively). The evenness of Actinobacteria and Betaproteobacteria in both N + C treatments, and of Gammaproteobacteria in N + C2, were each significantly lower than those in the N-only treatment ($F_{12} = -2.78$ to -4.28 , $p = 0.017$ to 0.001). In addition, marginally significant differences in evenness compared to N-only were observed for Firmicutes in N + C2,

Gammaproteobacteria in N + C1, and Bacteroidetes in N + C2 ($F_{12} = -2.01$ to -2.06 , $p = 0.067$ to 0.059). Significant positive cubic proportional abundance trends were detected for Bacteroidetes, Betaproteobacteria, Firmicutes, and Gammaproteobacteria ($F_{87-99} = 2.21$ to 3.33 , $p = 0.029$ to 0.001), whereas significant negative cubic proportional abundance trends were observed for Acidobacteria, candidate division WPS-1, Planctomycetes, Verrucomicrobia, Alphaproteobacteria, and Deltaproteobacteria ($F_{87-99} = -2.004$ to -4.22 , $p = 0.048$ to <0.001). Proportional abundances of Betaproteobacteria in N + C1, and Gammaproteobacteria in both N + C1 and N + C2, were significantly higher than those in the N-only treatment ($F_{12-99} = 2.82$ to 4.23 , $p = 0.008$ to <0.001). Conversely, proportional abundances of Acidobacteria in N + C2, and Planctomycetes and Verrucomicrobia in both N + C1 and N + C2, were significantly lower than those in the N-only treatments ($F_{12-99} = -2.67$ to -3.98 , $p = 0.009$ to 0.002).

Temporal biodiversity trends for Fungi

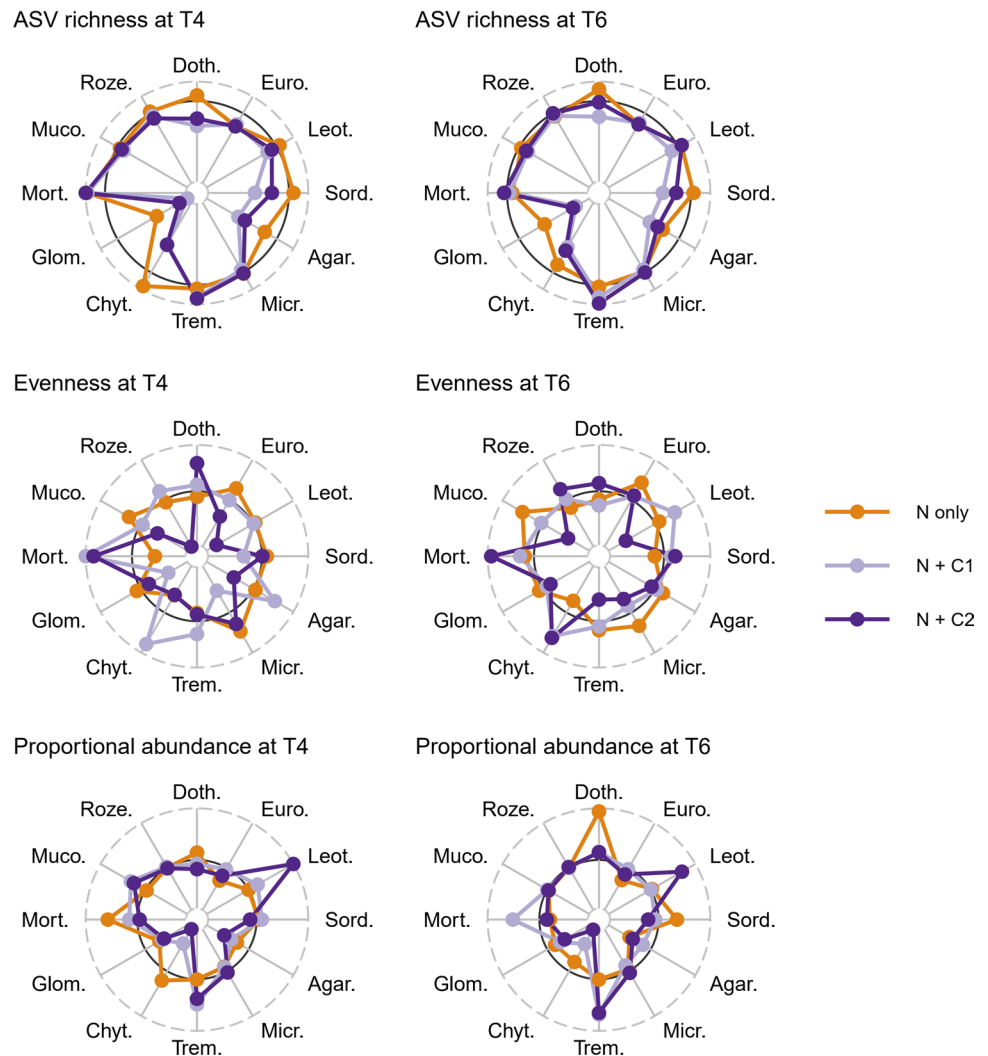
The mean richness of overall fungal communities declined to a significantly greater extent in the N + C2 treatment – by up to 134 ASVs (70%) after 28 days (T4) – than in the N-only treatment ($F_{12} = -2.45$, $p = 0.030$; Online Resource 2, Fig. S1c; Online Resource 2). Overall mean fungal evenness trends were similar to the richness trends but without significant treatment differences, whereas the proportional abundance trends differed little between treatments or times (Online Resource 2, Fig. S1e & f). Significant negative cubic and quadratic trends were detected for richness and evenness, respectively, but not proportional abundance, of overall fungal communities ($F_{87} = -4.23$ and 3.32 , $p \leq 0.001$; Online Resource 1).

Biodiversity trends for individual fungal taxa were more variable than those for bacterial taxa, most commonly reaching maximal divergence from initial values after 28 days (T4). The mean richness of Dothideomycetes, Leotiomyces, Sordariomycetes, Agaricomycetes, Chytridiomycota, Glomeromycota, and Rozellomycota were each lower in N + C treatments than in the N-only treatment, by varying extents and across various time spans between 14 (T3) and 56 (T5) days (Fig. 4 & Online Resource 2, Fig. S2a). Glomeromycota had the largest richness declines in all treatments, by 28 ASVs (48%) in N-only, and 44–49 ASVs (88–89%) in N + C treatments after 28 days (T4), while Chytridiomycota richness increased by 10 ASVs (47%) after 28 days in the N-only treatment but declined by 20 ASVs (80–91%) after 28 days in the N + C treatments. Conversely, Tremellomycetes richness increased, albeit by only 3 ASVs (86%) in the N-only treatment, and by 8–10 ASVs (183–200%) in the N + C treatments. After 112 days (T6), 11 and seven of 12 fungal taxa had lower mean richness than initial values in the

N + C1 and N + C2 treatments, respectively, by as much as 38 ASVs (76%) for Glomeromycota in N + C2. Significant negative cubic richness trends were detected for Agaricomycetes, Chytridiomycota, Dothideomycetes, Eurotiomycetes, Glomeromycota, and Rozellomycota ($F_{87} = -2.01$ to -4.81 , $p = 0.047$ to <0.001 ; Online Resource 1). Mean richness was significantly lower in N + C treatments compared to N-only for Chytridiomycota in both N + C1 and N + C2, and Sordariomycetes in N + C1 ($F_{12} = -2.83$ to -3.47 , $p = 0.015$ to 0.005), while a marginally significant difference was observed for Dothideomycetes in N + C1 only ($F_{12} = -2.17$, $p = 0.051$). Conversely, mean richness of Tremellomycetes was significantly higher in both N + C treatments compared to N-only ($F_{12} = 2.585$ and 3.788 , $p = 0.024$ and 0.003).

Evenness changed little in the N-only treatment for most fungal taxa, whereas clear evenness declines were observed in the N + C2 treatment for Leotiomyces (by 70% after 14 days) and Rozellomycota (by 77–80% after 14–56 days) (Fig. 4 & Online Resource 2, Fig. S3b). In contrast, Mortierellomycota evenness increased in the N + C treatments, by up to 145% after 28 days (T4) in N + C1. The evenness decline observed for Leotiomyces in the N + C2 treatment was mirrored by a large proportional abundance increase (up to 3,070% after 56 days) (Online Resource 2, Fig. S3c). Substantial proportional abundance increases in the N + C treatments over time were also observed for Tremellomycetes (up to 1,447% in N + C2 after 112 days), Mucoromycota (up to 609% in N + C1 after 28 days), Microbotryomycetes (up to 1,822% in N + C2 after 14 days; but 65% below initial values after 56 days), and Mortierellomycota (up to 504% in N + C1 after 7 days). In contrast, the mean proportional abundance of Chytridiomycota in the N + C treatments was clearly lower than values in the N-only treatment, declining by over 90% relative to initial values. Significant quadratic trends were detected for evenness of Leotiomyces and Rozellomycota, as were significant negative cubic trends for evenness of Glomeromycota and Mucoromycota ($F_{87} = -2.72$ to 2.82 , $p = 0.030$ to 0.006 ; Online Resource 1). Significant quadratic trends were detected for proportional abundances of Agaricomycetes and Leotiomyces, as were significant negative cubic trends for proportional abundances of Chytridiomycota, Eurotiomycetes, Glomeromycota, and Tremellomycetes ($F_{87} = -4.197$ to 3.395 , $p = 0.018$ to <0.001). Evenness was significantly lower in N + C treatments compared to N-only for Glomeromycota in N + C1, and Mucoromycota in both N + C1 and N + C2 ($F_{12-99} = -2.66$ to -2.71 , $p = 0.021$ to 0.009), whereas Chytridiomycota evenness was higher in N + C1 than in N-only with marginal significance ($F_{12} = 2.16$, $p = 0.052$). The mean proportional abundances of Dothideomycetes were significantly lower in both N + C1 and N + C2 treatments compared to the N-only treatment ($F_{12} = -2.554$ and -2.367 , $p = 0.025$ and 0.036 , respectively). Conversely, the mean proportional

Fig. 4 Mean ASV richness, evenness, and proportional abundances of the twelve most common fungal classes, 28 days (T4) and 112 days (T6) after applications of bovine urine with N at a rate of 700 kg N ha⁻¹ (N-only), or urine plus labile carbon (sucrose) at rates of 5.1 t C ha⁻¹ (N+C1) and 10.1 t C ha⁻¹ (N+C2), relative to values at T0, immediately before urine input. Solid circles represent T0 values, while grey dashed circles represent minimum and maximum biodiversity values at either T4 or T6. Taxonomic label abbreviations are as follows: Dothideomycetes; Eurotiomycetes; Leotiomycetes; Sordariomycetes; Agaricomycetes; Microbotryomycetes; Tremellomycetes; Chytridiomycota; Glomeromycota; Mortierellomycota; Mucoromycota; Rozellomycota



abundances of Leotiomycetes and Microbotryomycetes in N+C2, and Mortierellomycota in N+C1, were each significantly higher than those in the N-only treatment ($F_{12} = 2.351$ to 3.635, $p = 0.037$ to 0.003).

Effects of urine and carbon inputs on bacterial community function

A total of 230 biosynthesis pathways, 152 degradation/utilisation/assimilation pathways, and 48 precursor metabolite/energy generation pathways were detected using PICRUSt2 (Table 2; Online Resource 2, Figs S4-S6). Maximal pathway abundance changes within each treatment, both positive and negative, were typically observed after 14 or 28 days (T3 or T4). Pathway abundances subsequently approached but did not always return to their initial values by 112 days. A similar range of pathways was affected in each treatment, but the magnitude of pathway abundance changes was largest in the N+C2 treatment and smallest in the N-only treatment. For example, of the 230 biosynthesis pathways detected, 93 and

131 had increased and decreased abundances by means of 19.5% and -8.55%, respectively, in the N-only treatment after 14 days (T3), while 88 and 136 had increased and decreased abundances by means of 260% and -17.1%, respectively, in the N+C2 treatment (Table 2; Online Resource 2, Fig. S4). The largest biosynthesis pathway abundance increases were observed among the cell structure biosynthesis category, and the fatty acid and lipid biosynthesis category, among which 10 and eight pathways increased by means of 1,630% and 496%, respectively, after 14 days (T3) in the N+C2 treatment. In the N-only treatment, by comparison, eight pathways from each of these categories increased by means of only 13.1% and 5.61% at the same time point.

Most of the 152 degradation/utilisation/assimilation pathways detected temporarily increased in abundance in each treatment (Table 2; Online Resource 2, Fig. S5). Among carbohydrate degradation pathways, sucrose degradation III (sucrose invertase) and IV (sucrose phosphorylase) increased in abundance by 261% and 150%, respectively, after 14 days in the N+C2 treatment, compared to 21.4%

Table 2 Changes in bacterial metabolic pathway abundances after 14 and 112 days (T3 and T6) relative to T0. Values are mean percent abundance changes of pathways per category relative to T0, with the numbers of pathways with increased or decreased abundances shown in parentheses

Category	Time point	Change	N-only	N + C1	N + C2
Biosynthesis	14 days (T3)	Increase	19.49 (93)	131 (77)	259.97 (88)
		Decrease	-8.55 (131)	-13.23 (147)	-17.11 (136)
	112 days (T6)	Increase	39.37 (88)	40.46 (72)	35.44 (77)
		Decrease	-11.31 (136)	-16.45 (152)	-14.45 (147)
Degradation/Utilization/Assimilation	14 days (T3)	Increase	28.23 (106)	180.5 (103)	526.59 (108)
		Decrease	-17.56 (40)	-20.52 (45)	-19.2 (39)
	112 days (T6)	Increase	31.46 (105)	53.48 (101)	164.27 (108)
		Decrease	-20.52 (41)	-16.53 (47)	-15.28 (39)
Precursor Metabolite and Energy Generation	14 days (T3)	Increase	14.16 (28)	48.15 (28)	78.61 (29)
		Decrease	-18.3 (20)	-12.08 (20)	-12.63 (18)
	112 days (T6)	Increase	13.58 (23)	61.91 (23)	30.89 (27)
		Decrease	-14.14 (25)	-14.85 (25)	-7.55 (20)

and 2.51% at the same time point in the N-only treatment. Glucose/glucose-1-phosphate degradation and lactose/galactose degradation I pathway abundances increased by 152% and 334%, respectively, after 14 days (T3) in the N + C2 treatment, but increased by just 2.12% and decreased by -60%, respectively, at the same time point in the N-only treatment. Similarly, oxidative glucose degradation pathway abundance (secondary metabolite degradation) increased by 1,047% and 2,486%, respectively, in the N + C1 and N + C2 treatments after 14 days (T3), compared to only 52.8% at the same time point in the N-only treatment. Conversely, sucrose degradation II (sucrose synthase) pathway abundance increased by 93.7% after 14 days (T3) in the N-only treatment but declined by -23.9% at the same time point in the N + C2 treatment.

Among the precursor metabolite/energy generation pathways, photosynthesis and glyoxylate cycle pathway abundances both increased in all three treatments, by 22% and 43.8%, respectively, after 14 days (T3) in the N + C2 treatment, compared to 0.309% and 2.82%, respectively, in the N-only treatment (Table 2; Online Resource 2, Fig. S6). Similarly, three of four glycolysis pathways and five of seven TCA cycle pathways had increased abundances in all three treatments, by means of 69.4% and 54.7%, respectively, after 14 days (T3) in the N + C2 treatment, compared to 7.11% and 11%, respectively, in the N-only treatment.

Effects of urine and carbon inputs on fungal trophic modes and growth forms

Trophic modes and growth forms were assigned to 1,685 of 3,550 fungal ASVs (47%) from the FungalTraits database. The most abundant trophic modes detected were saprotrophs (67.6% of total FungalTrait-matched ASV abundance), parasites (19.6%), pathogens (9.33%), mycorrhizae (6.87%), and endophytes (1.26%) (Fig. 5a). In the N-only treatment, saprotrophs (49.4 to 59.7%) and parasites (26.1

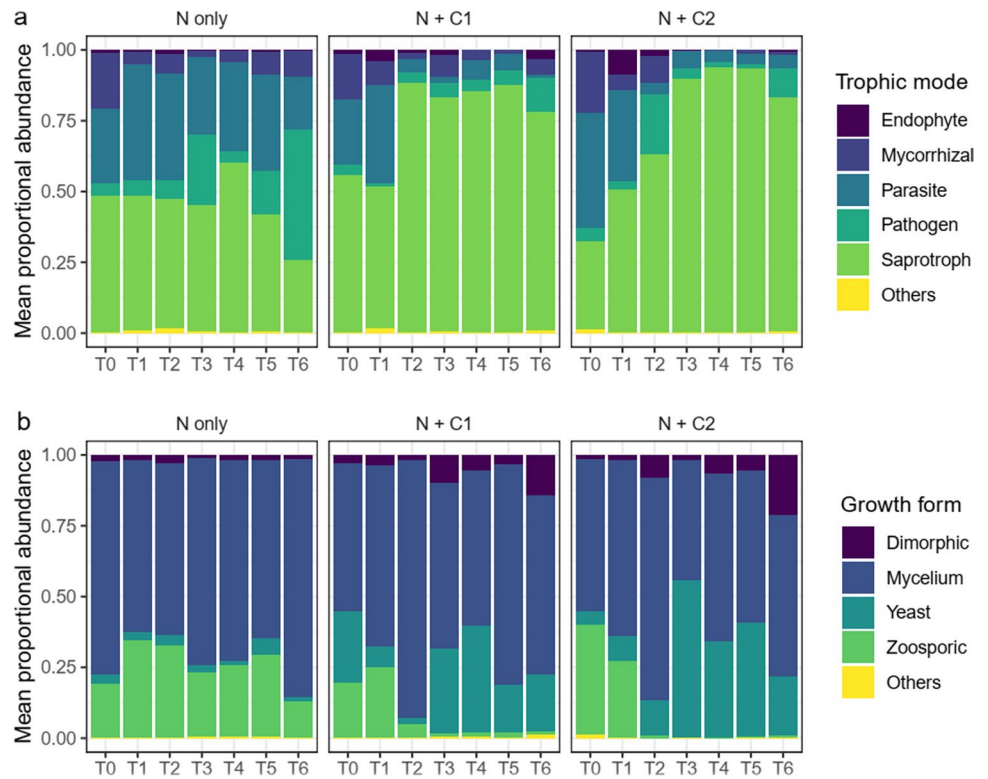
to 40.9%) together represented the largest mean proportions of trophic modes from T0 until 56 days (T5), but the proportion of pathogens (45.9%) exceeded that of both saprotrophs (25.6%) and parasites (18.9%) after 112 days (T6). Trophic mode composition in the N + C treatments became highly dominated by saprotrophs (77.9% to 94.7%) after 7 (N + C1) or 14 (N + C2) days. In all three treatments, the mean proportions of mycorrhizae dropped sharply from T0 values (16.1 to 21.6%) to 1 day (T1; 4.4% to 8.4%) and subsequent time points, reaching particularly small values ($\leq 1.3\%$) from 14 to 112 days in the N + C2 treatment.

The most common growth forms detected were mycelium (68%), followed by yeasts (16.9%), zoosporic (14.8%) and dimorphic (5.1%) fungi (Fig. 5b). Mycelia accounted for the largest mean proportions of growth forms at all time points in all three treatments, except for 42% after 14 days (T3) in the N + C2 treatment. Proportions of zoosporic growth forms ranged from 34.3% after 1 day (T1) to 12.6% after 112 days (T6) in the N-only treatment, and from 19.6% to 38.9% before 7 days (T0-T1) in the N + C treatments. The proportions of zoosporic growth forms then dropped sharply, to $< 2\%$ from 14 (T3) to 112 (T6) days in the N + C treatments. These were largely replaced by yeasts, which represented 16.7% to 37% (N + C1) and 20.9% to 55.6% (N + C2) of growth forms from 14 to 112 days (T3-T6). The mean proportions of dimorphic ASVs were $\leq 3.1\%$ at all time points in the N-only treatment, but reached maximal values of 14.4% and 21.1% after 112 (T6) days in the N + C1 and N + C2 treatments, respectively.

Discussion

Recent research has demonstrated that inputs of labile carbon, as sucrose, to pastoral soil reduces the availability and leaching of nitrogen (Talbot et al. 2019). This is attributed to carbon-stimulated microbial growth and associated

Fig. 5 Changes in the mean relative abundances of fungal (a) trophic modes and (b) growth forms over time in response to applications of bovine urine with N at a rate of 700 kg N ha⁻¹ (N-only), or urine plus labile carbon (sucrose) at rates of 5.1 t C ha⁻¹ (N + C1) and 10.1 t C ha⁻¹ (N + C2). The “Others” category consists of trophic modes/growth forms represented by less than 1% of the total abundance of ASVs matching the FungalTraits database. T0 through T6 correspond to samples collected immediately before, and 1, 7, 14, 28, 56 and 112 days after application of urine. Carbon was added two days before T0



immobilisation of nitrogen. In this study, we show that these carbon inputs have substantial effects on the biodiversity, composition, and function of microbial communities in the soil. Our data suggests the addition of labile carbon may support the recovery of bacterial communities from a simulated bovine urination event, but is likely to worsen the impact of this event on fungal communities.

Our experiment involved repeated sampling of soil communities over several months, thereby capturing succession processes operating at timescales of days to months, driven by differing abilities amongst bacterial and fungal taxa to utilise various resources in the soil. The observed biodiversity shifts will partly reflect seasonal changes, but the magnitude of changes of biodiversity metrics quantified in this study were larger in response to urine combined with labile carbon inputs than to urine alone, clearly showing that carbon inputs substantially altered the composition and function of soil communities over this time scale.

The differing responses of bacterial and fungal communities are likely due to their contrasting biological characteristics. Broadly, soil fungi have growth and turnover rates up to an order of magnitude slower than prokaryotes (Rousk and Bååth 2011). Furthermore, hydrophilic carbon forms, such as the sugar applied in this case, tend to be rapidly immobilised by fast-growing soil bacteria, whereas hydrophobic carbon fractions tend to be immobilised more slowly by fungi (Deng et al. 2021). Therefore, bacteria are likely to have outcompeted slower-growing fungi for labile carbon

uptake and growth. However, fungi include both slower-growing and faster-growing, more adaptable taxa, with urea inputs having been shown to favour the latter (Ganasamurthy et al. 2021). In this study, carbon inputs adversely affected mycorrhizal, non-saprotrophic, and zoospore fungi, but promoted the growth of saprotrophs, especially fast-growing yeasts. These results indicate that inputs of labile carbon to reduce nitrogen leaching from urine deposits (Talbot et al. 2019) also likely change the function of fungal communities. Below, we consider the effects of these carbon inputs on bacterial and fungal communities in more detail.

Effects of urine and carbon inputs on bacterial biodiversity

Overall bacterial ASV richness declined substantially, and to similar extents, following urine (N-only) and urine plus labile carbon (N + C) inputs, recovering to approximately initial levels after 112 days in all three treatments. These trends can be attributed to temporary adverse impacts of urine deposits on soil microbes, including sudden increases in osmotic stress and soil pH (Petersen et al. 2004; Orwin et al. 2010; Bertram et al. 2012). Richness recovers as urine inputs dissipate and their adverse effects lessen, and as organisms begin to utilise urine-derived resources, as well as sugar in the N + C treatments, for growth. That bacterial ASV richness recovered by the experiment end in each treatment suggests these communities are resilient to bovine

urine and carbon inputs at the timescales considered in this experiment.

However, multivariate analyses indicated that bacterial community composition at the end of the experiment differed from the initial composition in all three treatments. Indeed, examination of ASV turnover throughout the experiment showed that substantial minorities of initially detected ASVs were no longer detected at subsequent time points. These losses were offset by varying proportions of newly detected ASVs, to a greater extent in the treatments receiving both urine and labile carbon than in the urine-only treatment. Consequently, bacterial communities in all three treatments had somewhat altered composition just 1 day after urine application, and did not return to the initial composition for at least 112 days, despite no further urine (or carbon) inputs. That the greatest shifts occurred between 1 and 7 days (T1-T2) suggests that the experimental treatments were the main drivers of changing soil community composition, but the failure of bacterial communities to entirely return to their initial compositions after 112 days (T6) might be due to seasonal effects.

While overall bacterial richness trends did not differ significantly between treatments, bacterial evenness trends in the urine plus carbon treatments differed significantly from that in the urine-only treatment, temporarily declining by up to 10%. These changes, especially from 7 to 56 days, show that these communities became temporarily more dominated by fewer organisms, presumably those most able to utilise labile carbon (in the form of sucrose) for growth. Furthermore, proportional abundance of overall bacterial communities showed a temporarily declining trend by up to 60% in response to urine-only inputs, but steady to slightly increasing trends throughout the experiment in response to urine and carbon inputs. While this visible difference between trends was not statistically significant, this is consistent with the premise that carbon inputs stimulate growth of bacterial populations and promote nitrogen immobilisation.

ASV richness trends for different bacterial taxa showed similar richness responses to urine-only and urine plus carbon inputs as overall bacterial communities. In all cases except for Verrucomicrobia, carbon inputs somewhat reduced the extent of urine-caused richness declines, suggesting that labile carbon inputs offset the negative impacts of urine inputs for at least some ASVs from most of the examined taxa. Further insights into the effects of urine and carbon were provided by contrasting evenness and proportional abundance responses to these inputs observed among the different taxa. Trends of temporarily increased proportional abundance and decreased evenness in response to urine plus carbon inputs were observed for Actinobacteria, Bacteroidetes, Firmicutes, Betaproteobacteria, and Gammaproteobacteria. This implies that carbon inputs preferentially stimulated population growth among these taxa,

suggesting their involvement in nitrogen immobilisation and associated reductions in nitrate leaching observed in Talbot et al. (2019). The changes in evenness indicate that only subsets of the ASVs within each of these taxa had this response; these are likely to be taxonomically-clustered sets of organisms from certain taxonomic orders, as observed by Goldfarb et al. (2011). Conversely, several other taxa showed evidence of temporary carbon-related declines in abundance, with either unchanging or declining evenness. These included Acidobacteria, candidate division WPS-1, Deltaproteobacteria, Planctomycetes and Verrucomicrobia, suggesting that ASVs from these taxa may be negatively affected by labile carbon inputs, and did not contribute to microbial nitrogen immobilisation. A possible explanation for this is that fast-growing copiotrophic organisms (i.e. ASVs from those taxa showing carbon-related abundance increases) rapidly utilised the available sugar, depleting the resource pool available to slower-growing oligotrophs (Goldfarb et al. 2011).

These observed changes in the proportional abundances of different bacterial taxa in response to carbon inputs are largely in agreement with previous research on soil bacterial responses to carbon, adding to evidence that high-level bacterial taxa may have predictable ecological traits (Fierer et al. 2007; Philippot et al. 2010). For example, according to Fierer et al. (2007), labile carbon levels were positively correlated with abundances of Bacteroidetes and Betaproteobacteria, and negatively correlated with Acidobacteria abundance, suggesting these taxa may be classed as copiotrophic and oligotrophic, respectively. Similarly, Goldfarb et al. (2011) identified positive responses of Actinobacteria, Betaproteobacteria, and Gammaproteobacteria to labile carbon, along with negative responses of Acidobacteria, Bacteroidetes, families within Bacilli and Clostridia (Firmicutes), Deltaproteobacteria, and Planctomycetes. This is consistent with our results, except we observed carbon-related proportional abundance increases for Bacteroidetes (as did Fierer et al. 2007) and Firmicutes. One reason for these discrepancies might be that the trends observed in our study are due to the combination of carbon and urine amendments, rather than carbon alone. Another possibility might be that phylum/class-level trends conceal differing responses among organisms at lower taxonomic levels (Goldfarb et al. 2011), and differing subsets from these taxa are included in each study. However, 80% of our Firmicutes ASVs were Bacilli and Clostridia, among which most families had negative responses to sugar according to Goldfarb et al. (2011).

Effects of urine and carbon inputs on fungal biodiversity

Fungal communities were more strongly and negatively impacted by urine and labile carbon inputs than bacterial

communities were. While overall fungal ASV richness declined in all three treatments, the largest decline, by up to 70%, was observed in the N + C2 treatment. Overall fungal richness did not recover to initial values after 112 days in any of the three treatments. Indeed, most fungal ASVs detected at the start of the experiment in all three treatments were no longer detected after 7 to 14 days. In the N-only treatment these lost ASVs were largely replaced by newly detected ASVs, but this was not the case after combined addition of urine and labile carbon. This demonstrates a severe adverse impact of labile carbon inputs on the biodiversity of soil fungi communities, over the time frame considered in this experiment.

Seven out of 12 examined fungal classes had somewhat reduced ASV richness and proportional abundance values, particularly from 14 or 28 days onward, in response to urine plus carbon inputs compared to urine inputs alone. While not always significant, these differences suggest that negative effects of labile carbon on fungal communities in soil are taxonomically widespread. The largest declines in richness and proportional abundance were observed for Glomeromycota (in response to all treatments) and Chytridiomycota (in response to labile carbon but not urine alone). The latter taxon, more commonly studied in aquatic systems, includes both parasites and saprotrophs that break down recalcitrant carbohydrates (Medina and Buchler 2020). Consequently, the observed depletion of Chytridiomycota may be due to labile carbon being an unfavourable nutritional resource for saprotrophic organisms within this taxon. Dothideomycetes and Sordariomycetes, among others, showed similar but less pronounced declining richness trends to Chytridiomycota, which may similarly be due to the depletion of recalcitrant carbohydrate decomposers within these taxa in response to labile carbon inputs.

Not all fungi were adversely affected by labile carbon inputs, however. A substantial decline in mean fungal evenness was observed in the N + C2 treatment, exceeding that observed for bacterial communities in the same treatment (but not significantly differing from the N-only or N + C1 trends). This decline suggests that the higher level of labile carbon input tended to favour a subset of fungal organisms that consequently became more dominant within the community. Only one fungal class, Tremellomycetes, showed a modest but clear richness increase in response to inputs of urine plus carbon, but not urine inputs alone. This taxon includes a variety of morphological forms and life cycles about which we have a limited ecological knowledge (Weiss et al. 2014). A handful of ASVs from this class with moderate to high proportional abundances from 28 days onward in the N + C treatments, but not detected at the experiment start nor in the N-only treatment, included *Apiotrichum*, *Holtermanniella*, *Naganishia*, and *Solicoccozyma* spp. *Apiotrichum* and *Solicoccozyma* species have been identified

as important organisms in forest soils (Buée et al. 2009; Mašínová et al. 2017), probably related to the presence of plant-derived labile carbon resources. Species within the genera *Solicoccozyma*, *Holtermanniella*, and *Naganishia* have previously been identified as *Cryptococcus* spp. (Liu et al. 2015), possibly suggesting that labile carbon inputs to soil may favour the growth of organisms that are closely related to those associated with mammalian disease.

In addition to these richness changes, a significant carbon-related evenness decline and proportional abundance increase was observed for Leotiomycetes, indicating that a subset of organisms within this ecologically diverse taxon benefited from the labile carbon inputs. Indeed, an ASV identified as *Tetracladium* sp., which was not detected at any time in the N-only treatment, or within the first 7–14 days of the experiment in the N + C treatments, was in contrast the first or second most abundant of all fungal ASVs after 28 days in the N + C2 treatment. The top five most abundant ASVs detected after 28 days in N + C2 also included *Scutellinia* sp. (Pezizomycetes), *Candida* sp. (Saccharomycetes), and *Holtermanniella* sp. (Tremellomycetes). None of these ASVs were detected in the N-only treatment, or at the experiment start in the N + C treatments, indicating that labile carbon is a strongly favourable resource for these organisms. All of these taxa, along with many of the fungal ASVs with observed large richness and proportional abundance increases in response to carbon inputs, are yeasts. These increases may partly reflect morphogenic switching from filamentous to yeast growth forms (Nadal et al. 2008), prompted by the seasonal increase in soil temperature or perhaps changing resource availability.

Effects of urine and carbon inputs on bacterial and fungal functional composition

Changes to bacterial metabolic pathway categories inferred via PICRUSt2 (Douglas et al. 2020) in the N + C treatments indicate alterations to processes underlying bacterial carbon utilisation, growth and associated nitrogen immobilisation. Observed increases in the abundances of pathways involved in the degradation of carbohydrates, including sucrose and glucose, are clearly related to utilisation of the labile carbon inputs. Glycolysis, glyoxylate, and TCA cycle pathways are components of core metabolism and energy production, with increases in the abundances of these pathways thus indicating increased bacterial activity and growth. Positive and negative changes in the abundances of various biosynthesis pathways, such as those associated with production of amino acids, fatty acids, lipids, and cell structure components, may be due to a combination of changes in the range of microbes that are growing, repair of cell damage caused by urine inputs, and a shift from a relatively stable to an actively growing state in response to increased labile

carbon availability. These pathway abundances returned towards their initial values by the end of the experiment, suggesting that bacterial metabolic functions are not affected in the long term by urine or labile carbon inputs. Identification of fungal trophic modes and growth forms based on the fungalTraits database (Pölme et al. 2020) showed that inputs of urine and labile carbon resulted in a profound shift in the proportion of saprotrophs from about half of fungal ASVs at the experiment start to almost all remaining ASVs from 14 or 28 days until the experiment end. These remaining saprotroph ASVs are likely to be those that can utilise labile carbon for growth, including fast-growing yeasts and dimorphic fungi, as shown by increased proportions of ASVs with these growth forms from 14 days after urine and carbon inputs. The depletion of ASVs with non-saprotrophic trophic modes suggests a loss of fungal ecological functions in response to urine and labile carbon inputs. In particular, the apparent depletion of mycorrhizal growth modes (corresponding to Glomeromycota) may have negative implications for nutrient uptake by plants (George et al. 1995) and control of soil pathogens (Azcón-Aguilar and Barea 1997), with consequences for agricultural productivity and fertiliser requirements. Furthermore, zoospore fungi (corresponding to Chytridiomycota) consume detritus and organic material, and are in turn consumed by protists and metazoans (Gleason et al. 2012). Therefore, the observed losses of these organisms in response to labile carbon inputs suggests changes within the microbial food web. Furthermore, although we cannot directly compare abundances of bacterial and fungal communities, the more negative impacts of labile carbon inputs on fungal communities compared to bacterial communities might suggest a decreased biomass ratio of the former to the latter, which may cause reduced potential for carbon sequestration within these soils (Bailey et al. 2002; Malik et al. 2016). The trophic and growth mode composition of fungal communities barely recovered after 112 days, suggesting potentially long-term impacts of labile carbon inputs on fungal ecosystem services.

Limitations and considerations for nitrogen leaching

DNA from non-living microbes likely contributes to the communities and functions detected in this study (Carini et al. 2016). Metatranscriptomic analyses of gene expression would therefore be required to confirm whether our observations of changing pathway abundances are indicative of active microbial functions. In addition, inferences of organism abundances based on metabarcoding data are confounded by PCR biases (Pinto and Raskin 2012; Elbrecht and Leese 2015), and further qPCR measurements would be needed to confirm abundance changes among different taxa. Nonetheless, we observed clear trends of changing

biodiversity, proportional abundance, and function of soil communities, in response to labile carbon inputs.

This experiment involved a single simulated bovine urination event. In a functioning agricultural system, urination events will occur frequently and repeatedly at multiple locations. Labile carbon may therefore need to be repeatedly applied across large areas to achieve meaningful reductions in nitrate pollution. Such widespread inputs of carbon are likely to have wider effects on soil biogeochemical processes. In particular, current knowledge of soil priming suggests that these inputs are likely to lead to the release of more soil carbon to the atmosphere as CO₂ (Bastida et al. 2019). Furthermore, adverse effects on soil fungal communities would be replicated throughout space and time, leading to widespread and ongoing effects throughout the pastoral ecosystem. This points to a trade-off between reductions in nitrogen leaching and consequences for soil biodiversity and function. Inputs of labile carbon to soil can reduce nitrogen leaching, but this appears to be at the expense of fungal biodiversity and function in pastoral soils, with potential consequences for soil food webs and the carbon cycle.

Conclusions

Inputs of labile carbon to reduce nitrate losses reduced the impacts of a simulated bovine urine event on soil bacterial communities. In contrast, these carbon inputs adversely affected fungal communities for a period of at least several months, potentially altering soil food web dynamics and ecosystem functions such as carbon sequestration. Further research is required to tease apart the responses of lower taxonomic ranks to labile carbon inputs, to confirm the effects of these inputs on soil community functions and food webs, and to assess their impacts on soil biogeochemistry and the carbon cycle.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00374-023-01710-y>.

Acknowledgements We thank William Talbot and Keith Cameron for establishment of the field experiment, Graeme Rogers for help with the collection of soil samples, and Steven McNeill for valuable statistical advice.

Author contributions SAW, DW, and G-AG developed the research concept, acquired project funding, and planned the field experiment. CD collected the soil samples and carried out molecular laboratory procedures. AD analysed the data. AD wrote the manuscript, with input from G-AG.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions This work was funded by the New Zealand Ministry of Business, Innovation and Employment (MBIE) Endeavour Fund for the programme ‘Reducing nitrogen losses from farms’ led by Manaaki Whenua – Landcare Research (contract C09X1610) and

Manaaki Whenua – Landcare Research’s Strategic Science Investment Fund.

Data availability The sequence data generated in this study is available from GenBank SRA under Bioproject PRJNA891052. Processed metabarcoding data and analysis code used to generate the results in the paper are available from the Manaaki Whenua – Landcare Research Datastore (<https://datastore.landcareresearch.co.nz/en/dataset/ashleydene-lysimeter-experiment-1>).

Declarations

Competing interests The authors have no competing interests to declare.

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