


Changes in microbial utilization and fate of soil carbon following the addition of different fractions of anaerobic digestate to soils

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Abstract

Applying digestate, the residue from anaerobic digestion, to soil as a replacement for inorganic fertiliser is of growing interest in agriculture. However, the impacts of different fractions of digestate on the soil carbon (C) cycle remain unclear and provide the focus for the research reported here. We examined the effects of applying whole digestate (WD) and solid digestate (SD) on carbon dioxide (CO₂–C) efflux, the concentrations of dissolved organic carbon (DOC), microbial biomass C (C_{micro}) and phospholipid fatty acids, alongside carbon use efficiency (CUE). A 21-day laboratory microcosm incubation was used to investigate the impacts of digestate when applied to two grassland soils of high versus low initial nutrient content. Application rates for SD and WD were based on recommended nitrogen (N) inputs to grassland soils for these organic materials. Compared to control treatments, cumulative CO₂–C efflux and the concentration of DOC increased significantly after WD and SD application, although only within the low nutrient soil. Both C_{micro} and the fungal-to-bacterial ratio increased significantly following SD application, regardless of the initial soil nutrient content. These observations are likely to reflect the larger input of C, alongside the dominance of more strongly lignified compounds, associated with SD compared to WD to achieve a constant N application rate. Our results also indicate that the two digestate fractions generated significantly different CUE. The application of SD led to increases in C_{micro} and positive values of CUE, whereas decreases in C_{micro} and negative values of CUE were observed following WD application. These findings emphasize the need to carefully plan the management of digestate in agricultural production systems, to minimize negative impacts on C storage within soils whilst maximizing the agronomic value derived from digestate.

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Highlights

- Past research has not fully elucidated the impacts of digestate fractions on the soil C cycle.
- Soil nutrient status + digestate fraction shown to impact microbial community and CO₂—C efflux.
- Solid digestate fraction has positive impacts on microbial biomass and carbon use efficiency.

KEYWORDS

carbon cycle, carbon dioxide flux, carbon use efficiency, digestate, microbial community, microbial respiration, soil nutrient status

1 | INTRODUCTION

Agricultural soil is the largest active terrestrial reservoir in the global carbon (C) cycle. However, some agricultural practices, including deep tillage, over-application of inorganic fertilisers and intensification, have significantly impacted soil structural, chemical and biological conditions, increasing carbon dioxide (CO₂) emissions from soil and reducing soil organic matter (SOM) content (FAO, 2017). In contrast, soil C stocks may be increased by the promotion of agricultural practices that sequester soil organic C (FAO, 2017; Rumpel & Kögel-Knabner, 2011), through fixing atmospheric CO₂ within soil following plant photosynthesis and the transfer of CO₂ to plant biomass, or through the addition of allochthonous organic matter to soil. Additional practices may also help to reduce the environmental impacts of agricultural production, including crop rotation, improved nutrient and water application practices and the reduction of tillage intensity (IPCC, 2014). However, due to microbial metabolism, the application of organic materials to agricultural soil may also result in the release of significant quantities of CO₂, methane (CH₄) or nitrous oxide (N₂O) to the atmosphere (WRAP, 2016).

Interest in the application of digestate, the residue remaining after anaerobic digestion, to agricultural soil has grown substantially given the potential agronomic value of this material. Digestate generally has a low C-to-N ratio (C:N), is rich in NH₄⁺, P, K⁺, Na⁺, Mg²⁺ and other macronutrients, and can improve soil structure, water infiltration rate and water-holding capacity (García-Albacete, Tarquis, & Cartagena, 2014; Möller & Müller, 2012; Tambone et al., 2010). However, there are significant uncertainties surrounding the impact of digestate application on the C cycle within agricultural soils. This is particularly true following solid-liquid separation and the application of different fractions of digestate to soil. Separation allows for differentiation of the total nutrient content of digestate into individual phases, enhancing the potential to match digestate

application to crop nutrient requirements when compared with the whole fraction of digestate without separation (Marcato, Pinelli, Pouech, Winterton, & Guisresse, 2008). Whole digestate is a mixture of fibre and liquid, with high viscosity and low infiltration potential. It is generally rich in N, P, K⁺ and other macronutrient elements that are present in plant-available forms and usually has a C:N < 10 (Tambone et al., 2010). In contrast, the solid fraction is rich in total P (up to 90% of total P in whole digestate may be retained in the solid fraction), much present as water extractable P, alongside Ca²⁺, Mg²⁺, S and Mn, usually with a C:N > 10 (Bachmann, Uptmoor, & Eichler-Löbermann, 2016; Hjorth, Christensen, Christensen, & Sommer, 2010; Lukehurst, Frost, & Seadi, 2010; Marcato et al., 2008; Panuccio, Attinà, Basile, Mallamaci, & Muscolo, 2016). The forms of organic C present in the whole and solid fractions of digestate can also differ substantially. The whole fraction has been shown to be a mixture of dissolved organic carbon (DOC), which is readily available to microorganisms after application to land, and lignin compounds. In contrast, the solid fraction is dominated by recalcitrant organic C compounds, including lignin, cutin, humic acids and other complex compounds, considered as humus precursors with high biological stability (Nkoa, 2014; Tambone, Genevini, D'Imporzano, & Adani, 2009) that can promote SOM accumulation.

The application of digestate as a fertiliser in agriculture may influence C metabolism by the soil microbial community, which biosynthesizes the C into compounds for growth and/or emits CO₂ through respiration. This balance dictates the carbon use efficiency (CUE), which may be defined as the efficiency of the biosynthesis of organic C from a source material relative to its respiration (Manzoni, Taylor, Richter, Porporato, & Ågren, 2012). Usually, when CUE is positive and high the soil microbial community utilizes a C source for biosynthesis and growth, favouring the anabolic pathway, leading to C stabilization in soil. In contrast, when CUE is low and/or negative, microbial utilization of a C source for biosynthesis is less efficient, the

catabolic pathway is favoured, respiration rate and CO₂ production are enhanced and C sequestration in soil is reduced (Geyer, Kyker-Snowman, Grandy, & Frey, 2016; Wang & Post, 2012; Wang, Post, & Mayes, 2013). Many factors influence the CUE, including temperature, moisture, quality of the C source (e.g., C:N) and nutrient availability in soil. For example, Sinsabaugh, Manzoni, Moorhead, and Richter (2013) reported that application of an organic material to soil that is rich in recalcitrant C (often C:N > 20), such as the solid fraction of digestate, can increase bacterial catabolism in order to produce extracellular enzymes to hydrolyse C compounds and, consequently, CO₂ is produced. In contrast, the addition of organic matter with C:N < 20 to soil, such as the whole fraction of digestate, can promote bacterial biosynthesis of C and, consequently, reduce CO₂ production.

Soil nutrient availability, particularly the concentrations of N and P, may also influence CUE. When soil is not N or P limited relative to C (e.g., low soil C:N), CUE tends to increase because bacteria seek to maintain a balanced intracellular composition between C and nutrients (Manzoni et al., 2012; Roller & Schmidt, 2015) and thus microbial biomass concentration tends to increase. However, when an organic material containing liable C (e.g., the whole fraction of digestate) is applied to a low-nutrient soil (high soil C:N ratio and, potentially, N limitation) (Blagodatskaya, Blagodatsky, Anderson, & Kuzyakov, 2014; Moorhead & Sinsabaugh, 2006), bacteria tend to respire C that has been applied because maintenance respiration is increased. This is also true after application of poor-quality resources (e.g., recalcitrant compounds, such the solid fraction of digestate) to a stressed environment (e.g., low nutrient availability, high temperature or low water availability), because there is an increase in the cost of producing intra/extracellular catabolism under these conditions and an increase in CO₂ production (Malik, Puissant, Goodall, Allison, & Griffiths, 2019; Sinsabaugh, Hill, & Follstad Shah, 2009). Further, bacteria and fungi within the soil microbial community have potentially different effects on CUE. For example, fungi are able to degrade organic material with high C:N without emitting CO₂—C, thereby maintaining a high CUE, whereas bacteria are less efficient at degrading organic material with high C:N (Blagodatskaya & Kuzyakov, 2008). For bacteria, CUE also differs between *r* (growth strategists; high CUE) and *K* (competitive strategists; low CUE) communities (Keiblinger et al., 2010; Roller & Schmidt, 2015).

However, the impacts of digestate on the soil C cycle via microbial effects on CUE remain poorly understood, especially when different physical fractions of digestate with varying nutrient form and stoichiometry are applied to soils. The differing composition of whole and solid digestate may

influence soil bacterial and fungal communities differently, with potential effects on C cycling and CUE. There has also been insufficient research focussed on the interactions between digestate application and soil nutrient status, which has been considered as one of the main drivers influencing bacterial and fungal activity and, subsequently, soil C stocks and other soil health parameters. In this context, the research reported here tested the following hypotheses: (a) for soil at lower initial nutrient status, the application of either WD or SD stimulates microbial respiration and reduces CUE to a greater extent than for soil at higher initial nutrient status, (b) at low or high soil nutrient status, the application of WD will stimulate microbial respiration and reduce CUE compared to SD, and (c) the application of SD increases the fungal-to-bacterial ratio in soils at both low and high initial nutrient status, when compared to WD.

2 | MATERIALS AND METHODS

2.1 | Soil sampling and initial characterization

Soils were sampled from two fields adjacent to a commercial biogas plant (Cockerham Green Energy Ltd, North-west England, UK; latitude: 53.972, longitude: −2.822) on 17 September 2018. The two fields were selected to provide contrasting initial soil nutrient properties (Table 1) as driven by the management history of each field. Topsoil to 15-cm depth was sampled from each field using a gouge auger and following a “W” sampling protocol (Natural England, 2008), in which samples from 20 points along a “W” were combined into a single integrated soil sample for each field. High nutrient soil (HN) was under grass production at the time of sampling and used for grazing and silage production during previous years. This field receives liquid digestate four times per year, with the last application occurring at the end of July 2018. The low nutrient soil (LN) was fallow grassland at the time of soil sampling and had never previously received digestate. Following collection and homogenization, soils were sieved through a 2-mm mesh and stored in sealed plastic bags at 4 °C until the incubations began.

2.2 | Digestate sampling and characterization

On 24 September 2018, whole and solid fractions of anaerobic digestate were collected from Cockerham Green Energy Ltd, following sampling protocols detailed by the Agriculture and Horticulture Development Board (2017), and stored at 4°C prior to the start of the incubations. Digestate

TABLE 1 Initial physicochemical characteristics of soils used in the microcosm incubations (mean values reported, ± 1 standard error in parentheses, $n = 3$)

Soil characteristics	High nutrient soil	Low nutrient soil
Bulk density (g cm^{-3})	1.54 (0.14)	1.48 (0.014)
pH water (1:5 w/v)	7.31 (0.035)	5.06 (0.018)
NO_3^- (mg kg^{-1} DW soil)	71.05 (0.51)	66.66 (0.32)
NH_4^+ (mg kg^{-1} DW soil)	0.47 (0.044)	1.94 (0.10)
Olsen P (mg kg^{-1} DW soil)	40.66 (1.18)	10.42 (1.10)
P index UK (Agriculture and Horticulture Development Board, 2017)	4	1
Water extractable total organic C (mg kg^{-1} DW soil)	228.61 (14.23)	61.43 (0.76)
Soil total C (mg C kg^{-1} DW soil)	50,298.14 (68.49)	31,817.73 (39.3)
Soil total N (mg N kg^{-1} DW soil)	4,396.73 (160.30)	2,363.93 (199.82)
TC:TN	11.46 (0.07)	13.68 (0.50)
DM (%)	73.06 (0.10)	75.49 (0.02)

Abbreviations: DM, dry matter; NH_4^+ , ammonium; NO_3^- , nitrate; P index, mg L^{-1} P Olsen; P, phosphorus; total C, total carbon, non-acidified analysis; total N, total nitrogen; Water total C, water extractable total organic carbon, acidified analysis.

from Cockerham Green Energy Ltd is fermented in a mesophilic, single-stage digester with a retention time of 50 days. The feedstock is livestock and poultry manure, co-digested with food waste, including wheat, potatoes, tea bags and whey. Whole digestate is unpasteurised and separated into liquid and solid fractions using a screw-press. The liquid fraction is collected in covered lagoons, whereas the solid fraction is stored in an uncovered open space. Whole digestate was sampled directly from the anaerobic digester before separation, whereas the solid fraction was sampled from material that had been stored for 7 days prior to collection. The two fractions of digestate were chosen to provide contrasting properties for the experiment (Table 2).

2.3 | Experimental design

A microcosm incubation was carried out between 8 and 30 October 2018, involving control (Ctr), whole digestate (WD) and solid digestate (SD) treatments. Each amendment was conducted in triplicate for both HN and LN soil types, with soil \times amendment combinations placed randomly in amber and Duran bottles inside a temperature-, pressure- and moisture-controlled room in the dark. The WD and SD

TABLE 2 Physicochemical characteristics of whole and solid digestate used in the microcosm incubations ($n = 1$)

Parameter in fresh weight (FW)	Whole digestate (WD)	Solid digestate (SD)
DM (%)	11.6	24.3
Organic matter (%)	8.36	84.3
pH (1:6 w/v)	8.18	8.20
TN (mg kg^{-1} FW)	8,500	4,836
$\text{NH}_4^+\text{-N}$ (mg kg^{-1} FW)	4,921	752.81
TP (mg kg^{-1} FW)	2,869	4,209
TC (mg kg^{-1} FW)	37,000	109,107
TC:TN	4.35	22.56

Abbreviations: DM, dry matter; $\text{NH}_4^+\text{-N}$, ammonium nitrogen; TC, total carbon; TN, total nitrogen; TP, total phosphorus.

amendments were added to soils inside separate glass containers in order to achieve the same N application rate ($170 \text{ kg N (as } \text{NH}_4^+\text{-N) ha}^{-1} \text{ year}^{-1}$), after the Agriculture and Horticulture Development Board (2017). This resulted in the addition of $c.12,500 \text{ mg kg}^{-1}$ dry weight (DW) soil of C for SD and 625 mg kg^{-1} DW soil of C for WD treatments to both soils. Digestate fractions were mixed thoroughly with soil and then subdivided into Duran (for respirometry) or amber bottles (destructive samples) prior to the incubation.

The moisture content of the soils was set at 50% water-holding capacity (WHC) using milliQ water ($>18.2 \text{ M}\Omega\cdot\text{cm}$ at 25°C). Control soils were left unamended without any digestate addition and only received milliQ water in order to maintain 50% WHC. Respirometry measurements were carried out using a Micro-Oxymax Respirometer (Columbus Instruments International Corp., Columbus, OH, USA), with an automated 20-channel closed circuit and with two empty bottles used as analytical blanks. For respirometry samples, the respirometer maintained a constant moisture content throughout the incubation. The concentration of CO_2 in the headspace of each Duran bottle was monitored at a partial pressure of $1,063.9125 \text{ hPa}$ and a temperature of $23 \pm 1^\circ\text{C}$, via a specialized GL 45 three-port connection at 2-h intervals, with emission rates of $\text{CO}_2\text{-C}$ and cumulative $\text{CO}_2\text{-C}$ expressed as a rate (mg C h^{-1}) and as a mass (mg C), respectively. In addition, a parallel set of destructive samples was prepared using amber bottles in order to monitor changes in soil properties through time. These destructive samples were analysed at 0, 1, 2, 3, 4, 7, 14 and 21 days (for the 21-day time-point, respirometry samples were destructively sampled). The moisture content of the destructive samples was checked daily by weighing the amber bottles without lids and adding

milliQ water to maintain 50% WHC. The destructive samples were placed inside the same dark controlled room as the respirometry samples.

2.4 | Soil analyses

Destructive soil samples were analysed for microbial biomass C (C_{micro}) and dissolved organic carbon (DOC). Additional samples were taken at 0 and 21 days for analysis of phospholipid fatty acid (PLFA) content. Extraction for C_{micro} was carried out following the chloroform fumigation method (Brookes et al., 1985; Vance, Brookes, & Jenkinson, 1987). Duplicate fresh soils were extracted with and without chloroform fumigation according to Brookes et al. (1985) and Vance et al. (1987) (1:5 w/v, 0.5 M K_2SO_4 , pH ~ 7 , filtered Whatman No 42). The determination of TC for the two sets of extracts was carried out using a TOC-L/TN Series Analyser (Shimadzu, Kyoto, Japan) based on a combustion-reduction method. Microbial biomass C was calculated as the difference in concentration between fumigated and unfumigated samples, with subsequent correction by K_{ec} for C evolved as CO_2 (Brookes et al., 1985; Joergensen, 1995, 1996).

Fresh soil samples were extracted in milliQ water (1:10 w/v; 15 min shaking) for DOC analyses (Jones & Willett, 2006), filtered (Whatman No 42) and the extract was analysed using a TOC-L/TN Series Analyser (Shimadzu) after sample acidification to remove inorganic C.

The PLFA extraction was carried out as described by Quideau et al. (2016), using a three-stage extraction. Frozen soil ($-80^\circ C$) was freeze-dried and between 1 and 1.5 g of soil was used for the extraction. Extracted samples were analysed using a Gas Chromatograph-FID (Agilent Technology 6890N, Santa Clara, CA, USA). A C13 (methyl tridecanoate) and C19 (methyl nonadecanoate) mixed standard was used as an internal standard in order to identify the range of the retention times of the PLFAs of interest.

Soil pH was determined on fresh soil samples (1:5 w/v; 30 min shaking) using milliQ water. Air-dried soil samples were analysed for Olsen P as described by Murphy and Riley (1962) and Olsen, Cole, Watanabe, and Dean (1954). Samples were extracted (1:20 w/v; 30 min shaking) with a 0.5 M $NaHCO_3$ solution, with pH adjusted to 8.5, and subsequently filtered (Whatman No 42). The extracted samples were analysed using a SEAL Autoanalyzer AA3 (Seal Analytical, Fareham, UK; Method No G-103-92 Rev1; Multitest Mt7/MT8) based on the molybdenum blue colorimetric reaction. Soil dry matter (DM) and loss-on-ignition (LOI) were determined using a gravimetric method (Allen, 1989; Gardner, 1986).

Approximately 12 g of fresh soil was oven-dried at $105^\circ C$ for 48 h to constant weight to determine DW. Subsequently, around 1.5 g of oven-dried soil was heated at $550^\circ C$ for 6 h in a muffle furnace, left to cool overnight and subsequently weighed to determine LOI. The TC (total carbon) and TN (total nitrogen) content of soils was determined using an automated Dumas procedure on a Carbo Erba NA 1500 analyser (Erba Science, Surrey, UK), working with 30 ± 1 mg of oven-dried and ball-milled soil. Fresh soil samples were also extracted for available N using 1 M KCl (1:5 w/v, 1 h shaking) (Bremner, 1965; McTaggart & Smith, 1993) and filtered (Whatman No 42). The filtrate was subsequently analysed for NH_4^+ and NO_3^- content using a SEAL Autoanalyzer AA3 (Seal Analytical; Method No G-102-93 Rev 2; Multitest MT7/MT8) with two different colorimetric reactions (ISO 11732, 1997 and ISO 13395, 1996, respectively).

2.5 | Calculations for % TC respired, CUE and statistical analysis

The % TC respired from soils after the addition of digestate was calculated as:

$$\begin{aligned} \%TC \text{ respired at each time} - \text{point} = & \\ & (\text{cumulative } CO_2 - C \text{ produced at each time} - \text{point} / \\ & (\text{TC present in the soil at day 0} \\ & + \text{TC applied in digestate amendment})) * 100, \end{aligned}$$

where all C terms were expressed in mg.

The CUE was estimated as described by Frey, Gupta, Elliott, and Paustian (2001) and Tiemann and Billings (2011), using the following equation:

$$CUE = dBc / (dBc + \sum CO_2 - C),$$

where dBc is the change in C_{micro} and $\sum CO_2 - C$ is the cumulative C lost through microbial respiration during the incubation, both expressed in mg C. For both WD and SD treatments, C_{micro} and $\sum CO_2 - C$ were standardized by the Ctr treatment, in order to focus on the fate of C that was added to the soil with digestate, following Tiemann and Billings (2011). The CUE of Ctr treatments was not calculated, because no C was added to soils.

Statistical analyses were performed in R version 3.6.1 (R Core Team, 2019). One-way and two-way analysis of variance (ANOVA) were employed to assess the significance of the factors “soil” (HN, LN) and “digestate amendment” (Ctr, WD, SD) and their interaction.

TABLE 3 Summary of one-way and two-way ANOVA results from microcosm incubations

	Soil			Digestate			Soil × digestate interaction			p-value	Standard error			p-value	Standard error		
	Mean	Standard error		Mean	Standard error		Mean	Standard error			Mean	Standard error			Mean	Standard error	
CUE	HN LN	−0.087 −0.025	0.038 0.013	n.s.	WD SD	−0.22 ^a 0.11 ^b	0.17 0.05	0.015						n.s.			
Cumulative CO ₂ —C (mg C kg ^{−1} DW soil)	HN	1,382.18 ^a	133.9	0.0009	Ctr	738.02 ^a	132.20	0.00002	HN		Ctr: 1247.67 ^{a,a}	228.53		0.00007			
	LN	942.62 ^b	125.3		WD	1,250.56 ^b	156.50				WD: 1323.85 ^{a,a}	239.73					
					SD	1,417.88 ^b	192.50				SD: 1413.04 ^{a,a}	275.16					
									LN		Ctr: 228.88 ^{a,b}	45.75					
% TC respired	HN	1.44	0.25	n.s.	Ctr	1.43 ^a	0.39	0.0002	HN		Ctr: 2.22 ^{a,a}	0.47		0.0001			
	LN	1.60	0.34		WD	2.66 ^b	0.38				WD: 2.06 ^{a,a}	0.43					
					SD	2.38 ^b	0.40				SD: 1.25 ^{a,a}	0.41					
									LN		Ctr: 0.63 ^{a,b}	0.14					
C _{micro} (mg kg ^{−1} DW soil)	HN	796.31 ^a	24.38	0.0007	Ctr	684.60 ^a	20.14	4.3*10 ^{−10}			WD: 3.21 ^{b,a}	0.63					
	LN	698.01 ^b	21.63		WD	663.18 ^a	22.78				SD: 3.18 ^{b,a}	0.71		n.s.			
					SD	891.38 ^b	30.93										
									LN		Ctr: 0.0030	0.0030		0.005			
Fungal-to-bacterial ratio	HN	0.11	0.0052	n.s.	Ctr	0.11 ^a	0.0031				WD: 0.11 ^a	0.0031		n.s.			
	LN	0.11	0.0035		WD	0.11 ^a	0.0031				SD: 0.13 ^b	0.0067					
					SD	0.13 ^b	0.0067										
									HN		Ctr: 157.12 ^{a,a}	15.68		0.000002			
DOC (mg kg ^{−1} DW soil)	HN	166.11 ^a	9.66	0.000002	Ctr	110.45 ^a	10.68	4*10 ^{−9}	HN		WD: 161.93 ^{a,a}	15.66					
	LN	117.03 ^b	10.15		WD	120.15 ^a	10.87				SD: 179.30 ^{a,a}	19.10					
					SD	194.12 ^b	12.72		LN		Ctr: 63.79 ^{a,b}	5.69					
											WD: 78.36 ^{a,b}	9.36					
											SD: 208.94 ^{b,a}	16.75					

Note: Columns from left to right describe effects of initial soil nutrient status (high [HN] vs. low [LN]); effects of digestate amendment (control [Ctr], whole digestate [WD], solid digestate [SD]); and interactions between soil nutrient status and digestate amendment. “n.s.” represents effects that were not statistically significant ($p > 0.05$). Tukey tests were employed to determine differences between individual levels of soil type and digestate amendment, with significant differences between levels denoted using superscript letters. For interactions between soil type and digestate amendment, first superscript letter represents differences between digestate amendments within each soil type, and second superscript letter represents differences between soil type within each digestate amendment.

Levene's tests were used to check the homogeneity of variance assumption of ANOVA, with \log_{10} or square root transformations applied to data where necessary. A Tukey-test (honest significant difference [HDS]) was employed to compare individual levels where a significant factor was identified in ANOVA. For CUE, a Kruskal-Wallis test was used to assess the significance of the factors soil type and digestate amendment.

Due to the non-linear nature of many response variables across the incubations, multivariate polynomial regression was used to model time \times soil type \times digestate amendment interactions. Time was treated as a numerical variable and expressed from 0 to 21 days. For C_{micro} and DOC, in order to fully capture the nonlinear nature of changes through time, a cubic polynomial regression was used, whereas for cumulative $\text{CO}_2\text{—C}$ efflux, %TC respired and fungal:bacterial ratio linear regression models were applied. Where significant regression models were identified, *t*-tests were performed on cumulative $\text{CO}_2\text{—C}$ efflux, %TC respired and fungal:bacterial data in order to determine the nature of the time \times soil type \times digestate amendment interaction.

In all statistical analyses, *p*-values <0.05 were deemed as significant, whereas *p*-values between 0.05 and 0.06 were marked as borderline significant after Hofmann and Meyer-Nieberg (2018). Residual plots (S-L, Q-Q, residual-leverage and Cook's distance - leverage) were employed to assess the quality of the model fits and the assumption of normally distributed residuals for ANOVA, as well as the presence of leverage points or outliers. Missing observations were excluded from the analysis and no data imputation was performed. Clear outliers, assumed to represent sample error or contamination, were removed from the datasets prior to analysis.

3 | RESULTS

3.1 | Influence of treatments on $\text{CO}_2\text{—C}$ efflux from soils

Cumulative $\text{CO}_2\text{—C}$ efflux from HN soils was significantly greater than from LN soils across the incubations ($p < 0.001$) (Table 3). Further, digestate amendment exerted significant control on cumulative $\text{CO}_2\text{—C}$ efflux ($p < 0.0001$), with higher cumulative $\text{CO}_2\text{—C}$ efflux observed after the application of digestate to soils compared to control treatments, in the order $\text{Ctr} < \text{WD} \approx \text{SD}$. However, an interaction between soil type and digestate amendment was observed ($p < 0.0001$), with significant increases in cumulative $\text{CO}_2\text{—C}$ efflux after WD and SD application only occurring within LN soils and not within HN soils.

A significant three-way interaction between time, soil type and digestate amendment was also observed for cumulative $\text{CO}_2\text{—C}$ efflux, as shown in Figure 1 ($p < 0.0001$). Within the LN soil, both WD and SD amendments increased cumulative $\text{CO}_2\text{—C}$ efflux rapidly and significantly through time when compared to the control treatment, reaching +563% (SD) and +377% (WD) at 21 days compared to fluxes in the control treatment. Further, SD and WD diverged significantly from each other from 14 days onwards. Within the HN soil, only the SD amendment generated significantly higher cumulative $\text{CO}_2\text{—C}$ efflux and only from 14 days of the incubation onwards (+20% at 21 days when compared with Ctr), whereas WD and Ctr did not differ significantly.

Figure 2 reports the percentage of TC present in the combination of soil and digestate amendment that was respired as $\text{CO}_2\text{—C}$ during the incubations. In contrast to cumulative $\text{CO}_2\text{—C}$ efflux, no significant difference in %TC respired was observed between HN and LN soils. However, both WD and SD amendments resulted in significant increases in %TC respired compared to the Ctr ($p < 0.001$), in the order $\text{Ctr} < \text{WD} \approx \text{SD}$. Further, a significant interaction between soil and digestate amendment ($p < 0.001$) indicated that significant increases in %TC respired following SD or WD application only occurred in the LN soil, consistent with observations related to cumulative $\text{CO}_2\text{—C}$ efflux.

A highly significant three-way interaction between time, soil type and digestate amendment was observed ($p < 0.0001$), indicating that the temporal pattern in %TC respired after the addition of digestate depended on the nature of the soil at the start of the incubation. In the HN soil, digestate amendments followed the same temporal trend as the Ctr treatment. However, in the LN soil the %TC respired increased significantly through time following both WD (+372% at 21 days) and SD (+369% at 21 days) applications compared to the control treatment, an effect that was observed from 1 day onwards in the incubations.

3.2 | Influence of digestate amendments on the soil microbial community

Microbial biomass C was significantly higher in HN compared to LN soil ($p < 0.001$). Further, C_{micro} increased significantly after the application of SD compared to either Ctr or WD treatments ($p < 0.0001$), by +29% at 21 days in the HN soil and by +36% at 21 days in the LN soil compared to the Ctr treatment (Figure 3). No significant interactions between soil type, digestate amendment or time were observed for C_{micro} , confirming that the significant increase following the application of SD was observed in both HN and LN soils and throughout the duration of the incubations.

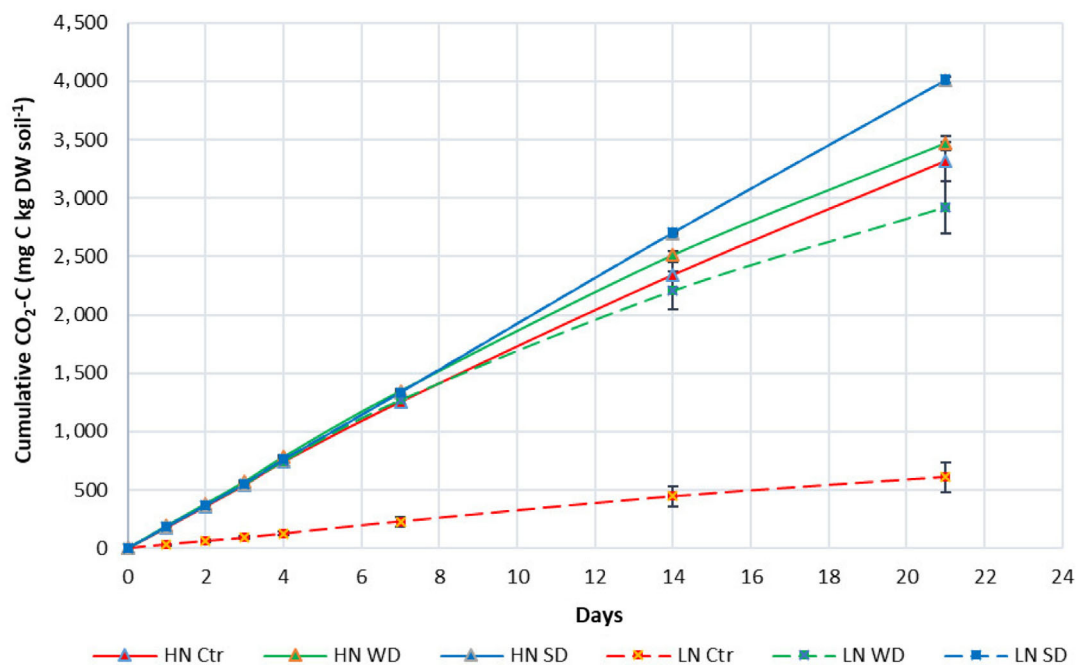


FIGURE 1 Cumulative CO₂-C produced from control (Ctr) soils or after addition of whole (WD) or solid (SD) fractions of digestate in soils with high (HN) or low (LN) initial nutrient status. HN × SD and LN × SD overlapping in the figure. Error bars ± 1 standard error

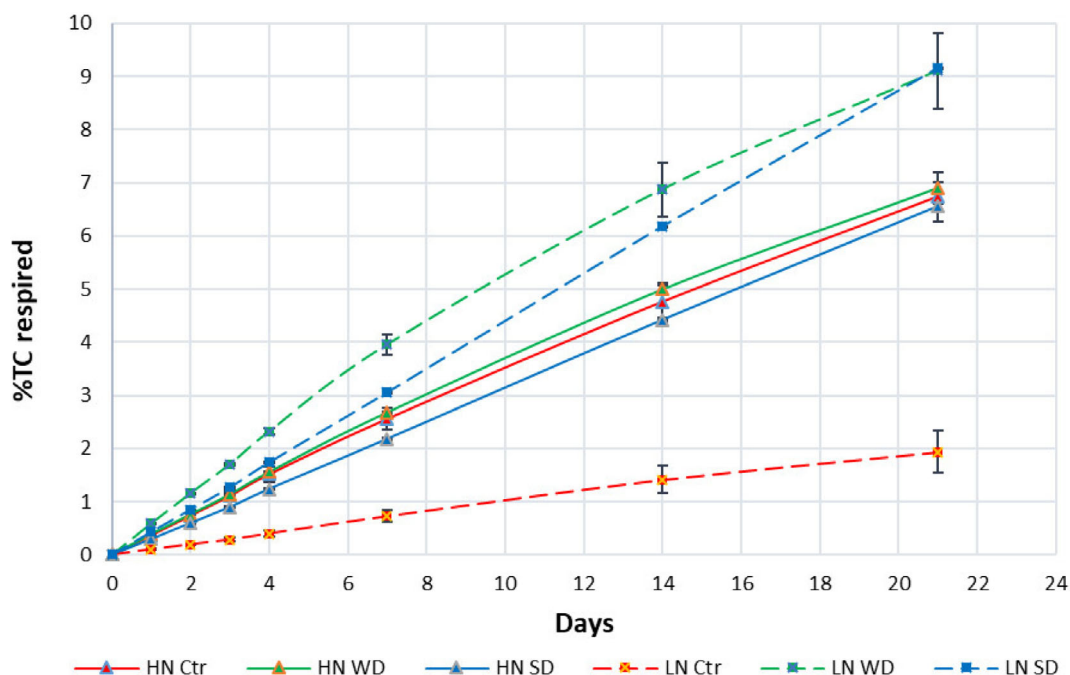


FIGURE 2 Percent total carbon (%TC) respired in control (Ctr) soils or after addition of whole (WD) or solid (SD) fractions of digestate in soils with high (HN) or low (LN) initial nutrient status. Error bars ± 1 standard error

Similarly to C_{micro} , the fungal-to-bacterial ratio increased significantly under the SD treatment compared to either the Ctr or WD treatments ($p < 0.01$), an effect that was also consistent across both HN and LN soils.

Further, time significantly affected the fungal-to-bacterial ratio (Figure 4), with a marginally significant three-way interaction observed between time, soil type and digestate amendment ($p < 0.049$). The fungal-to-

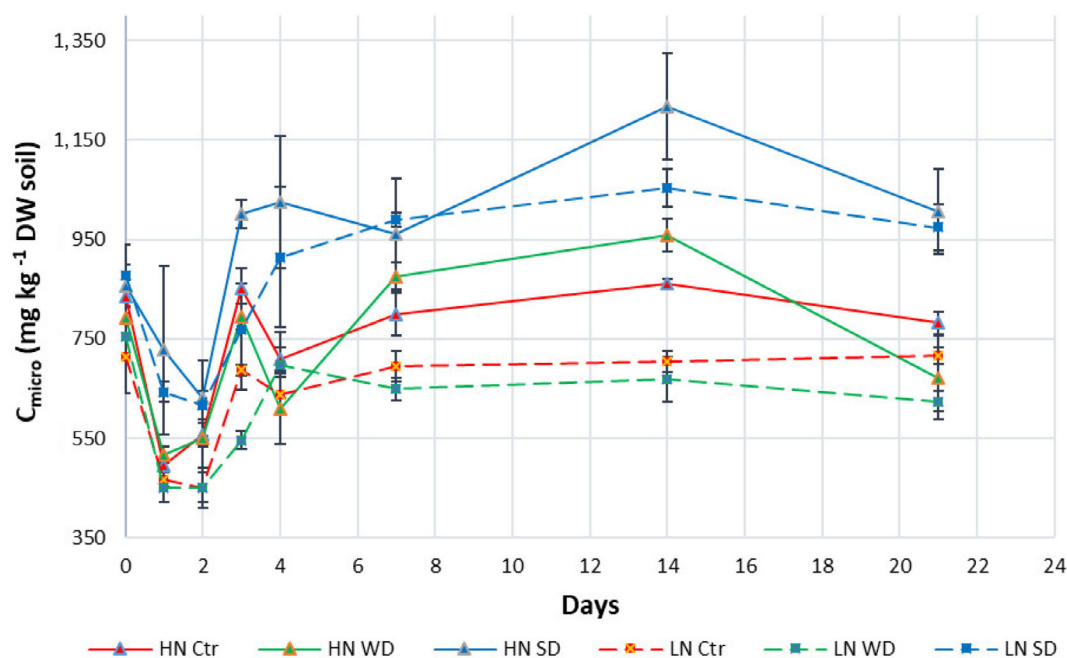


FIGURE 3 C_{micro} trends over time in control (Ctr) soils or after addition of whole (WD) or solid (SD) fractions of digestate with soils at high (HN) or low (LN) initial nutrient status. Error bars ± 1 standard error

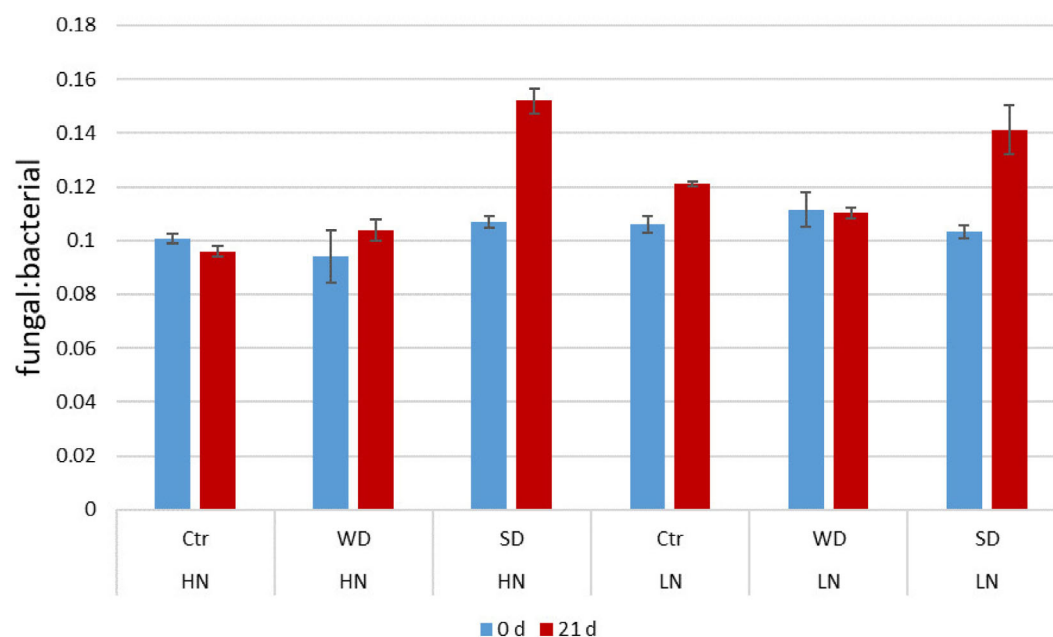


FIGURE 4 Fungal-to-bacterial ratio at 0 and 21 days in control (Ctr) soils or after addition of whole (WD) or solid (SD) fractions of digestate in soils with high (HN) or low (LN) initial nutrient status. Error bars ± 1 standard error

bacterial ratio increased significantly between 0 and 21 days following application of SD in both soils (+58% HN and +18% LN compared to Ctr), whereas the ratio decreased slightly (−8%) in the LN soil following the application of WD compared to the control ($p = 0.05$).

3.3 | Influence of digestate amendments on dissolved organic carbon concentration

The concentration of water-extractable DOC was significantly higher in HN compared to LN soils ($p < 0.0001$). Further, the application of SD to soils resulted in a

significant increase in the concentration of water-extractable DOC, compared to either WD or Ctr treatments ($p < 0.0001$). However, the impact of SD application differed between soil types, with a significant increase in DOC concentration following SD application only observed in the LN soil (Figure 5). No interaction between time, soil type and digestate amendment was observed with respect to DOC concentration.

3.4 | Estimation of CUE after digestate amendment

Table 4 reports the CUE for each combination of soil type and digestate amendment used within the incubation reported here. No significant difference in CUE was observed between the two soil types. However, digestate amendment exerted significant control on CUE ($p < 0.05$), with positive values of CUE observed following the application of SD and negative values after application of WD to soils; these effects were consistent across the two soil types used in the incubations.

4 | DISCUSSION

The application of digestate strongly influenced the C cycle within the soils examined during this research. This

was evidenced by significant changes in the loss of C via gaseous pathways, the production of water-soluble DOC, and the biomass and composition of the soil microbial community. However, for many parameters the impact of digestate application depended on the initial soil nutrient status, on the physical fraction of digestate that was applied, and on time across the 21 days incubation. It should be noted that the history of soil management within the HN and LN soils is likely to have driven different responses between these soils to the treatments applied in the experiments reported here. For example, past digestate application to the HN soil may have been responsible for differences in microbial community composition and functional traits, compared to the LN soil. Further, our experimental system did not include the input of labile

TABLE 4 Carbon use efficiency (CUE) following whole (WD) and solid fraction (SD) digestate amendments in high nutrient (HN) or low nutrient (LN) soils (mean values reported, ± 1 standard error in parentheses, $n = 3$)

Amendment	Estimation of CUE
HN \times WD	−0.37 (0.33)
HN \times SD	0.20 (0.050)
LN \times WD	−0.07 (0.035)
LN \times SD	0.02 (0.042)

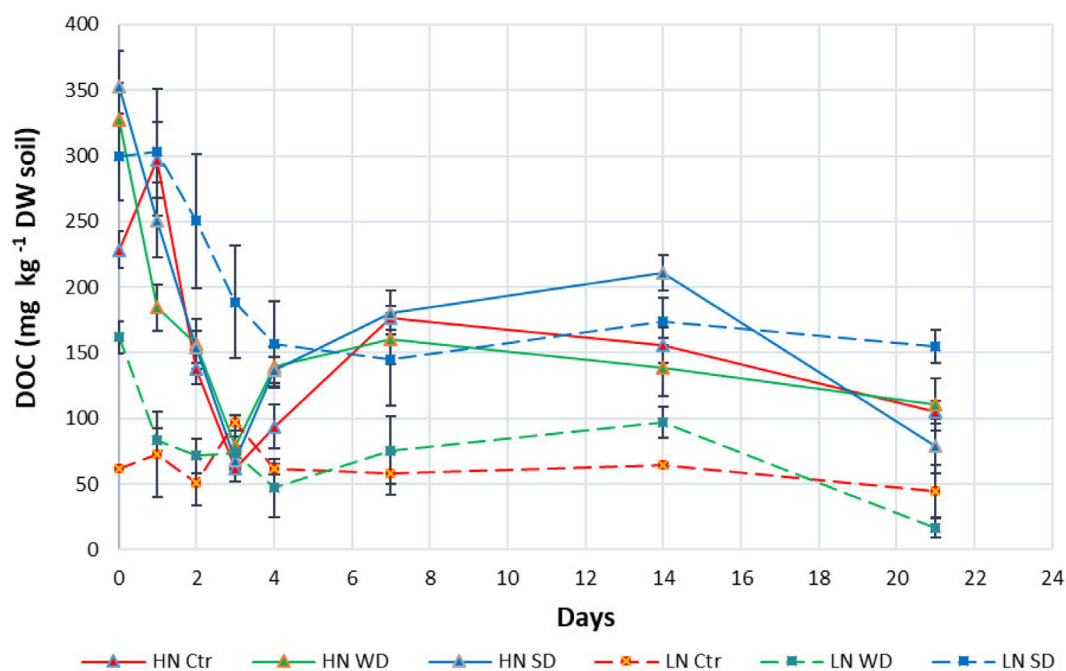


FIGURE 5 Dissolved organic carbon trends through time in control (Ctr) soils or after addition of whole (WD) or solid (SD) fractions of digestate in soils with high (HN) or low (LN) initial nutrient status. Error bars ± 1 standard error

C to soil from root exudates that may alter microbial requirements for digestate-derived C. Future research will be required in order to examine the interactions within plant-microbial-soil systems, including the net impacts of these interactions on the fate of C derived from inputs of digestate to agricultural soil, and the impacts of a wider range of soil management histories.

4.1 | The influence of digestate application on CO₂—C efflux

The efflux of CO₂—C from soil, whether expressed as an absolute flux or as a proportion of the TC within the combination of soil and digestate, increased significantly following the application of digestate. This observation is consistent with both previous laboratory and field research (e.g., Johansen, Carter, Jensen, Hauggard-Nielsen, & Ambus, 2013; Pezzolla et al., 2012; WRAP, 2016), spanning grassland and arable soils. For example, field experiments have reported an increase in cumulative CO₂ efflux occurring across a 12-month period following four whole digestate applications (WRAP, 2016) and across a 5-month period following three applications of whole digestate (Pezzolla et al., 2012). Further, a 9-day laboratory experiment on arable soil revealed a two-fold increase in cumulative CO₂—C efflux after whole digestate addition when compared with untreated soil (Johansen et al., 2013). Although the research we report above used digestate from a single feedstock, it should also be noted that some past research has demonstrated significant effects on CO₂ efflux associated with variation in digestate feedstock and post-digestion processing (i.e., separation) techniques (e.g., Askri, Laville, & Tre, 2016). These variables were not incorporated within the experimental system used in the research reported here.

The data reported above confirm that CO₂—C efflux was influenced by a significant interaction between soil type and digestate, in which increases in this gaseous flux of C following either WD or SD application only occurred in the LN soil. Increases in CO₂—C efflux following digestate application are partly consistent with de la Fuente, Alburquerque, Clemente, and Bernal (2013) and Grigatti, Di Girolamo, Chincari, Ciavatta, and Barbanti (2011), who report mineralization rates after the application of different fractions of digestate and their effects on CO₂—C efflux. However, de la Fuente et al. (2013) and Grigatti et al. (2011) report higher CO₂—C efflux following the application of SD compared to WD, whereas in the research reported here CO₂—C efflux did not differ significantly between the two fractions of digestate. It should be noted that the research of de la Fuente et al. (2013) involved a calcareous soil with

nutrient content similar to the HN soils used in our research, whereas Grigatti et al. (2011) also used a soil more similar in nutrient content to the HN compared to LN soil used in the current research. Differences in soil type may help to explain why no significant difference in CO₂—C efflux was observed between SD and WD within the LN soil in the research reported above. However, further work would be required in order to understand why similar variation in CO₂—C fluxes after application of different fractions of digestate were not observed in the HN soils.

The efflux of CO₂—C increased rapidly from the early stages of the incubations following the application of either SD or WD to the LN soil, whether expressed as cumulative CO₂—C or as a percentage of TC present in the soil-digestate system. The effects of digestate application in the LN soil are likely to reflect the activation of dormant bacteria and stimulation of maintenance respiration after the application of either fraction of digestate (Mondini, Cayuela, Sanchez-Monedero, Roig, & Brookes, 2006). In the LN soil, rapid increases in bacterial catabolism are likely to have followed the application of WD due to the input of readily available DOC, suggesting that this C source may have been utilized quickly for enzyme production and maintenance of respiration within a few days after application, consistent with other research (e.g., Wang et al., 2013; Wang & Post, 2012). After exhaustion of readily available C in WD, bacteria may have started to mine SOM present in the soil to meet continued demand for nutrients (Fontaine et al., 2011; Fontaine, Bardoux, Abbadie, & Mariotti, 2004), or alternatively turnover of the bacterial community may have occurred through the course of the incubation (Blagodatskaya, Blagodatsky, Anderson, & Kuzyakov, 2007), consistent with negative CUEs following the application of WD. However, the increase in CO₂—C efflux was higher and more persistent following the application of SD to the LN soil, possibly because fungal degradation of recalcitrant C compounds in SD produced C by-products that were subsequently consumed by bacterial catabolism. Alternatively, bacteria may have invested directly in enzymatic degradation of recalcitrant C such as lignin within SD, as reported by Sierra (2012). In turn, this is likely to have resulted in prolonged increases in respiration and CO₂—C efflux, consistent with Fontaine, Mariotti, and Abbadie (2003), Sinsabaugh et al. (2013) and Winogradzky (1924).

In contrast, within the HN soil, only during the later stages of the experiment and only after SD application were increases in CO₂—C efflux observed, and only when CO₂—C was expressed as a cumulative flux rather than as a percentage of TC present in the system. Following exhaustion of readily available C during the earlier stages of the incubation, by-products from fungal or bacterial

degradation of recalcitrant C within SD are likely to have supported the higher efflux of $\text{CO}_2\text{—C}$ from bacterial respiration towards the end of the incubation (Six, Frey, Thiet, & Batten, 2006). In contrast, rapid exhaustion of readily available C, combined with the absence of an input of more recalcitrant C in WD, meant that $\text{CO}_2\text{—C}$ efflux under this treatment did not differ significantly compared to the control within the HN soil.

Varying effects of digestate application on $\text{CO}_2\text{—C}$ efflux between HN and LN soils are also likely to reflect differences in physicochemical conditions between the two soil types that influenced microbial metabolic responses to the input of resources within the digestate (e.g., Larsson, Vonstockar, Marison, & Gustafsson, 1995; Manzoni et al., 2012; Russell & Cook, 1995). Within the HN soil, existing neutral soil pH, higher C_{micro} , and DOC and lower C:N meant that the changes in microbial respiration following digestate input were relatively small compared to the control soil treatment. In contrast, the adverse soil conditions in the LN soil (low pH, C_{micro} , DOC and nutrient concentration) created an environment in which respiration of CO_2 from control soils was relatively low, and in which activation of dormant bacteria and subsequent increases in respiration followed the application of resources within both WD and SD (Mondini et al., 2006).

4.2 | Changes in the soil microbial community following digestate application

Both C_{micro} and the fungal to bacterial ratio increased significantly following the application of SD, a pattern that was consistent across both HN and LN soils. Increases in C_{micro} following the application of SD were likely to be driven by higher inputs of TC compared to the WD treatment, in order to achieve a consistent N application rate across both fractions of digestate. The additional input of C resources allowed greater opportunity for biosynthesis and the accumulation of C within new soil microbial biomass under the SD treatment. These observations related to C_{micro} are supported by other research that has examined the impact of digestate application on the soil microbial community. For example, de la Fuente et al. (2013) report increases in C_{micro} only 7 days after the application of SD, driven by the high TC applied to soil with this fraction of digestate. Further, Chen et al. (2012) carried out a 21-day incubation and report an increase in C_{micro} that was related to a shift from r-strategists to K-strategists in soil that received biogas residues.

The fungal-to-bacterial ratio of control HN and LN soils indicated a microbial community that was dominated by bacteria, consistent with other research focused

on agricultural grasslands (Bardgett, Frankland, & Whittaker, 1993; Bardgett, Hobbs, & Frostegård, 1996; Bardgett & Leemans, 1995). However, this ratio increased significantly following the application of SD to both soils used in the incubations reported here, driven by an increase in fungal PLFA rather than a decrease in bacterial PLFA. This observation is likely to reflect the significant input of more recalcitrant C compounds, such as lignin, associated with SD compared to WD (Nkoa, 2014). Hydrolysis of these C compounds has been shown to rely predominantly on the action of fungi rather than bacteria (Hammel, 1997), consistent with the increase in total fungal PLFA through the incubations reported here following the application of SD and in agreement with other research (e.g., Rousk & Bååth, 2011; Walsh, Rousk, Edwards-Jones, Jones, & Williams, 2012). Fungal-produced C by-products following degradation of recalcitrant C within SD may also have sustained bacterial production (e.g., Bugg, Ahmad, Hardiman, & Rahmanpour, 2011; Dashtban, Schraft, Syed, & Qin, 2010; Ruttimann, Vicuna, Mozuch, & Kirk, 1991), including through generating a flush of DOC, which is available for the microbial community (Möller, Miller, & Kjöllér, 1999). In contrast, the limited input of recalcitrant C following WD application produced no significant change in fungal-to-bacterial ratio within the HN soil, alongside a relatively small and marginally significant decrease in this ratio within the LN soil, reflecting a decrease in total fungal PLFA within the microbial community under this treatment.

Although the concentration of DOC was significantly greater in soil following the application of SD compared to either Ctr or WD treatments, this effect was only observed within LN and not within HN soils. Within the HN soil, DOC generated following the application of SD appeared to be efficiently metabolized by the microbial community, evidenced by an increase in C_{micro} but no increase in $\text{CO}_2\text{—C}$ efflux compared to control soils. In contrast, the application of SD to the LN soil increased DOC concentrations by the end of the incubation. This is likely to reflect unfavourable conditions for the microbial community within the LN soil, including low pH and nutrient availability, which can limit microbial metabolism of DOC, as noted in previous research (David, Vance, Rissing, & Stevenson, 1989; Guggenberger, Glaser, & Zech, 1994; Jardine, Weber, & J. F. M., 1989; Vance & David, 1989).

4.3 | Changes in CUE following digestate application

Carbon use efficiency varied significantly between the digestate treatments used in the experiments reported here,

with consistent patterns observed across both soil types. The application of WD resulted in negative values of CUE, driven by greater decreases in C_{micro} and by increased $\text{CO}_2\text{--C}$ fluxes compared to control treatments during the incubations. Decreases in C_{micro} may reflect grazing by protozoa and/or microbial turnover (Frey et al., 2001). The input of readily degradable C substrates within WD is likely to have promoted the catabolic pathway and maintenance of respiration of bacteria to a greater extent compared to the anabolic pathway, resulting in enhanced $\text{CO}_2\text{--C}$ effluxes and decreased biosynthesis of C within microbial cells (Geyer et al., 2016; Manzoni et al., 2012). The magnitude of the effect of WD on CUE was more pronounced in HN compared to LN soils. This observation reflects the smaller cumulative $\text{CO}_2\text{--C}$ efflux in HN soils compared to the respective controls, generating a more negative value of CUE following the application of WD. Although C_{micro} also decreased following the application of WD to LN soils, the relatively large increase in $\text{CO}_2\text{--C}$ efflux compared to control soils resulted in a smaller value of CUE for LN soils compared to the HN soils. These observations emphasize the potential for application of WD to result in net decreases in C_{micro} , rather than net accumulation of C within soil microbial biomass, due to the stimulation of maintenance respiration and associated utilization of C from both native soil and substrate pools (e.g., Blagodatskaya et al., 2014; Moorhead & Sinsabaugh, 2006).

In contrast to WD, positive values of CUE were observed following the application of SD to both soil types, with CUE in the range 0–0.55, as reported for soil microbial communities by Sinsabaugh et al. (2013), who accounted for substrate C:N, the assimilation efficiency of N, bacterial C:N and a CUE_{max} in their research. However, it is notable that a higher CUE was observed after application of SD to HN compared to LN soils, reflecting substantial increases in C_{micro} and relatively small increases in cumulative $\text{CO}_2\text{--C}$ efflux in HN soils following SD application, compared to control soils. Although C_{micro} also increased in LN soils after the application of SD compared to control soils, the increases in $\text{CO}_2\text{--C}$ efflux were far more pronounced, resulting in lower values of CUE compared to HN soils. Increase in C_{micro} following SD application to soils indicates the potential for net accumulation of C within soil microbial biomass, in particular associated with increases in soil fungal community anabolism and biomass (Keiblinger et al., 2010). However, it should also be recognized that cumulative $\text{CO}_2\text{--C}$ fluxes following the application of SD exceeded those under all other treatments used in our experiments. Therefore, application of SD to soils can potentially generate adverse effects on absolute fluxes of CO_2 to the atmosphere, whilst at the same time contributing positively to the accumulation of C within soils.

5 | CONCLUSIONS

The research reported here provides important new insights into how changes in the soil C cycle may follow the application of digestate to agricultural grasslands. The precise nature of these impacts is contingent on the physical fraction of digestate applied to land and on the nutrient status of the soils that receive digestate. The solid fraction of digestate drove substantial increases in $\text{CO}_2\text{--C}$ efflux, an effect that appears to be inversely related to soil nutrient status. Microbial biomass C and the fungal-to-bacterial ratio in soil also increased following the application of the solid fraction of digestate, regardless of initial soil nutrient status. The effects of applying whole digestate to soil were more variable. Although $\text{CO}_2\text{--C}$ efflux increased following the application of whole digestate to soil at low initial nutrient status, no significant changes in microbial biomass C or in fungal-to-bacterial ratio followed the application of whole digestate. Carbon use efficiency in soils receiving solid digestate was positive, indicating the potential for C accumulation within soil microbial biomass. However, the accumulation of C within soil was exceeded by the additional C lost from soils via $\text{CO}_2\text{--C}$ efflux. Further, CUE was negative in both soil types following treatment with whole digestate, driven by decreases in C stored within microbial biomass and loss of C as $\text{CO}_2\text{--C}$.

These findings emphasize the need to carefully plan the management of digestate in agricultural production systems, in order to minimize negative impacts on C storage within soils whilst maximizing the agronomic value derived from digestate. Future research should seek to examine the impacts of a broader range of digestate fractions (whole, liquid, solid) on the soil C cycle in long-term field experiments, including the effects of plant–soil interactions and longer-term changes in CUE and SOM. In addition, research should seek to quantify the impacts of digestate application on other environmental parameters of concern, including the emission of greenhouse gases beyond CO_2 and the potential leaching of pollutants into the subsurface.

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AUTHOR CONTRIBUTIONS

Marta Cattin: Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing-original draft; writing-review & editing. **Kirk Semple:** Conceptualization; funding acquisition; supervision. **Marc Stutter:** Conceptualization; funding acquisition; investigation; supervision; validation; visualization; writing-review & editing. **Gaetano Romano:** Software; validation. **Alfonso Lag-Brotons:** Conceptualization; data curation; methodology; supervision. **Chris Parry:** Funding acquisition; resources. **Ben Surridge:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; supervision; validation; visualization; writing-review & editing.

CONFLICT OF INTEREST

The authors declare no relevant conflict of interest with respect to the content of this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Agriculture and Horticulture Development Board (2017). Nutrient Management Guide (RB209), section 2: Organic Material (May). <https://ahdb.org.uk/knowledge-library/rb209-section-2-organic-materials>.
- Allen, S. E. (1989). Chemical analysis of ecological materials. 2nd Edition, Blackwell Scientific Publications, Oxford and London.
- Askri, H. S., Laville, A. P., & Tre, A. (2016). Influence of origin and post-treatment on greenhouse gas emissions after anaerobic digestate application to soil. *Waste Biomass Valorisation*, 7, 293–306. <https://doi.org/10.1007/s12649-015-9452-6>
- Bachmann, S., Uptmoor, R., & Eichler-Löbermann, B. (2016). Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. *Scientia Agricola*, 73(1), 9–17. <https://doi.org/10.1590/0103-9016-2015-0069>
- Bardgett, R. D., & Leemans, D. K. (1995). The short-term effects of cessation of fertiliser applications, liming and grazing on microbial biomass and activity in a reseeded upland grassland soil. *Biology and Fertility of Soils*, 19(2–3), 148–154. <https://doi.org/10.1007/BF00336151>
- Bardgett, R. D., Frankland, J. C., & Whittaker, J. B. (1993). The effects of agricultural management on the soil biota of some upland grasslands. *Agriculture, Ecosystems and Environment*, 45 (1–2), 25–45. [https://doi.org/10.1016/0167-8809\(93\)90057-V](https://doi.org/10.1016/0167-8809(93)90057-V)
- Bardgett, R. D., Hobbs, P. J., & Frostegård, Å. (1996). Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils*, 22(3), 261–264. <https://doi.org/10.1007/BF00382522>
- Blagodatskaya, E., Blagodatsky, S., Anderson, T. H., & Kuzyakov, Y. (2014). Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. *PLoS One*, 9(4), e93282. <https://doi.org/10.1371/journal.pone.0093282>
- Blagodatskaya, E., & Kuzyakov, Y. (2008). Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. *Biology and Fertility of Soils*, 45(2), 115–131. <https://doi.org/10.1007/s00374-008-0334-y>
- Blagodatskaya, E. V., Blagodatsky, S. A., Anderson, T. H., & Kuzyakov, Y. (2007). Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Applied Soil Ecology*, 32(1–2), 95–105. <https://doi.org/10.1016/j.apsoil.2007.05.002>
- Bremner, J. M. (1965) Inorganic Forms of Nitrogen. In: Methods of Soil Analysis, Part 2, Agronomy Monograph No. 9, ASA and SSSA, Madison, 1179–1237.
- Brookes, P. C., Landman, A., Pruden, G., & Jenkinson, D. S. (1985). Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, 17(6), 837–842. [https://doi.org/10.1016/0038-0717\(85\)90144-0](https://doi.org/10.1016/0038-0717(85)90144-0)
- Bugg, T. D. H., Ahmad, M., Hardiman, E. M., & Rahmanpour, R. (2011). Pathways for degradation of lignin in bacteria and fungi. *Natural Product Reports*, 28(12), 1883–1896. <https://doi.org/10.1039/c1np00042j>
- Chen, R., Blagodatskaya, E., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., & Kuzyakov, Y. (2012). Decomposition of biogas residues in soil and their effects on microbial growth kinetics and enzyme activities. *Biomass and Bioenergy*, 45, 221–229. <https://doi.org/10.1016/j.biombioe.2012.06.014>
- Dashtban, M., Schraft, H., Syed, T. A., & Qin, W. (2010). Fungal biodegradation and enzymatic modification of lignin. *Journal Biochemistry and Molecular Biology Education*, 1(1), 36–50.
- David, M. B., Vance, G. F., Rissing, J. F., & Stevenson, F. J. (1989). Organic carbon fractions in extracts of O and B horizons from a New England spodosol: Effect of acid treatment. *Journal of Environmental Quality*, 18, 212–217. <https://doi.org/10.2134/jeq1989.00472425001800020014x>
- de la Fuente, C., Albuquerque, J. A., Clemente, R., & Bernal, M. P. (2013). Soil C and N mineralisation and agricultural value of the products of an anaerobic digestion system. *Biology and Fertility of Soils*, 49(3), 313–322. <https://doi.org/10.1016/j.agee.2011.03.007>
- FAO. (2017). *Soil organic carbon: the hidden potential*. Rome, Italy: Food and Agriculture Organization of the United Nations. <http://www.fao.org/3/a-i6937e.pdf>
- Fontaine, S., Mariotti, A., & Abbadie, L. (2003). The priming effect of organic matter: A question of microbial competition? *Soil Biology and Biochemistry*, 35(6), 837–843. [https://doi.org/10.1016/S0038-0717\(03\)00123-8](https://doi.org/10.1016/S0038-0717(03)00123-8)
- Fontaine, S., Bardoux, G., Abbadie, L., & Mariotti, A. (2004). Carbon input to soil may decrease soil carbon content. *Ecology Letters*, 7(4), 314–320. <https://doi.org/10.1111/j.1461-0248.2004.00579.x>
- Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J. M. G., Maire, V., ... Maron, P. A. (2011). Fungi mediate long term sequestration of carbon and nitrogen in soil through their

- priming effect. *Soil Biology and Biochemistry*, 43(1), 86–96. <https://doi.org/10.1016/j.soilbio.2010.09.017>
- Frey, S. D., Gupta, V. V. S. R., Elliott, E. T., & Paustian, K. (2001). Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. *Soil Biology and Biochemistry*, 33(12–13), 1759–1768. [https://doi.org/10.1016/S0038-0717\(01\)00101-8](https://doi.org/10.1016/S0038-0717(01)00101-8)
- García-Albacete, M., Tarquis, A. M., & Cartagena, M. C. (2014). Risk of leaching in soils amended by compost and digestate from municipal solid waste. *The Scientific World Journal*, 2014, 8–1768. [https://doi.org/10.1016/S0038-0717\(01\)00101-8](https://doi.org/10.1016/S0038-0717(01)00101-8)
- Gardner, W. H. (1986). Moisture content. In: *Methods of soil analysis. Part 1. Physical and mineralogical methods.*, 2nd edn, Soil Science Society of America. Book series 5, pp. 503–507.
- Geyer, K. M., Kyker-Snowman, E., Grandy, A. S., & Frey, S. D. (2016). Microbial carbon use efficiency: Accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry*, 127(2–3), 173–188. <https://doi.org/10.1007/s10533-016-0191-y>
- Grigatti, M., Di Girolamo, G., Chincari, R., Ciavatta, C., & Barbanti, L. (2011). Potential nitrogen mineralization, plant utilization efficiency and soil CO₂ emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy*, 35(11), 4619–4629. <https://doi.org/10.1016/j.biombioe.2011.09.007>
- Guggenberger, G., Glaser, B., & Zech, W. (1994). Heavy metal binding by hydrophobic and hydrophilic dissolved organic carbon fractions in a spodosol A and B horizon. *Water, Air, and Soil Pollution*, 72, 111–127.
- Hammel, K. E. (1997). Fungal degradation of lignin. *Driven by Nature: Plant Litter Quality and Decomposition*, 33–45. Wallingford: CAB International.
- Hjorth, M., Christensen, K. V., Christensen, M. L., & Sommer, S. G. (2010). Solid-liquid separation of animal slurry in theory and practice. A review. *Agronomy for Sustainable Development*, 30(1), 153–180. <https://doi.org/10.1051/agro/2009010>
- Hofmann, M., & Meyer-Nieberg, S. (2018). Time to dispense with the p-value in OR?: Rationale and implications of the statement of the American Statistical Association (ASA) on p-values. *Central European Journal of Operations Research*, 26(1), 193–214. <https://doi.org/10.1007/s10100-017-0484-9>
- IPCC. (2014). Climate change 2014: Mitigation of climate change. Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press.
- Jardine, P. M., McCarthy, J. F., & Weber, N. L. (1989). Mechanism of dissolved organic carbon adsorption on soil. *Soil Science Society of America*, 53, 1378–1385. <https://doi.org/10.2136/sssaj1989.03615995005300050013x>
- Joergensen, R. G. (1995). The fumigation-extraction method to estimate soil microbial biomass: Extraction with 0.01 M CaCl₂. *Agrobiological Research*, 48, 319–324.
- Joergensen, R. G. (1996). The fumigation-extraction method to estimate soil microbial biomass: Calibration of the K_{ec} value. *Soil Biology and Biochemistry*, 28(1), 25–31.
- Johansen, A., Carter, M. S., Jensen, E. S., Hauggard-Nielsen, H., & Ambus, P. (2013). Effects of digestate from anaerobically digested cattle slurry and plant materials on soil microbial community and emission of CO₂ and N₂O. *Applied Soil Ecology*, 63, 36–44. <https://doi.org/10.1016/j.apsoil.2012.09.003>
- Jones, D. L., & Willett, V. B. (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, 38(5), 991–999. <https://doi.org/10.1016/j.soilbio.2005.08.012>
- Keiblinger, K. M., Hall, E. K., Wanek, W., Szukics, U., Hämmerle, I., Ellersdorfer, G., ... Zechmeister-Boltenstern, S. (2010). The effect of resource quantity and resource stoichiometry on microbial carbon-use efficiency. *FEMS Microbiology Ecology*, 73(3), 430–440. <https://doi.org/10.1111/j.1574-6941.2010.00912.x>
- Larsson, C., Vonstockar, U., Marison, I., & Gustafsson, L. (1995). Metabolic uncoupling in *Saccharomyces cerevisiae*. *Thermochimica Acta*, 251(94), 99–110.
- Lukehurst, C., Frost, P., & Seadi, T. A. (2010). *Utilisation of digestate from biogas plants as biofertiliser*, IEA Bioenergy company, 1–36.
- Malik, A. A., Puissant, J., Goodall, T., Allison, S. D., & Griffiths, R. I. (2019). Soil microbial communities with greater investment in resource acquisition have lower growth yield. *Soil Biology and Biochemistry*, 132(November 2018), 36–39. <https://doi.org/10.1016/j.soilbio.2019.01.025>
- Manzoni, S., Taylor, P., Richter, A., Porporato, A., & Ågren, G. I. (2012). Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist*, 196(1), 79–91. <https://doi.org/10.1111/j.1469-8137.2012.04225.x>
- Marcato, C. E., Pinelli, E., Pouech, P., Winterton, P., & Guisresse, M. (2008). Particle size and metal distributions in anaerobically digested pig slurry. *Bioresource Technology*, 99(7), 2340–2348. <https://doi.org/10.1016/j.biortech.2007.05.013>
- McTaggart, I. P. & Smith, K. A. (1993). Estimation of potentially mineralisable nitrogen in soil by KCl extraction. *Plant and Soil*, 157(2), 167–174.
- Möller, J., Miller, M., & Kjoller, A. (1999). Fungal-bacterial interaction on beech leaves: Influence on decomposition and dissolved organic carbon quality. *Soil Biology and Biochemistry*, 31(3), 367–374. [https://doi.org/10.1016/S0038-0717\(98\)00138-2](https://doi.org/10.1016/S0038-0717(98)00138-2)
- Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in Life Sciences*, 12(3), 242–257. <https://doi.org/10.1002/elsc.201100085>
- Mondini, C., Cayuela, M. L., Sanchez-Monedero, M. A., Roig, A., & Brookes, P. C. (2006). Soil microbial biomass activation by trace amounts of readily available substrate. *Biology and Fertility of Soils*, 42(6), 542–549. <https://doi.org/10.1007/s00374-005-0049-2>
- Moorhead, D. L., & Sinsabaugh, R. (2006). A theoretical model of litter decay and microbial interaction. *Ecological Monographs*, 76(151–174), 151–174. [https://doi.org/10.1890/0012-9615\(2006\)076\(0151:ATMOLD\)2.0.CO;2](https://doi.org/10.1890/0012-9615(2006)076(0151:ATMOLD)2.0.CO;2)
- Murphy, J., & Riley, J. P. (1962). Determination single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31–36.
- Natural England (2008). Soil sampling for habitat recreation and restoration (February). Natural England Technical Information Note TIN035.
- Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. *Agronomy for Sustainable Development*, 34(2), 473–492. <https://doi.org/10.1007/s13593-013-0196-z>

- Olsen, S. R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. Washington, DC: U.S. Government Printing Office.
- Panuccio, M. R., Attinà, E., Basile, C., Mallamaci, C., & Muscolo, A. (2016). Use of recalcitrant agriculture wastes to produce biogas and feasible biofertilizer. *Waste and Biomass Valorization*, 7(2), 267–280. <https://doi.org/10.1007/s12649-015-9445-5>
- Pezzolla, D., Bol, R., Gigliotti, G., Sawamoto, T., López, A. L., Cardenas, L., & Chadwick, D. (2012). Greenhouse gas (GHG) emissions from soils amended with digestate derived from anaerobic treatment of food waste. *Rapid Communications in Mass Spectrometry*, 26(20), 2422–2430. <https://doi.org/10.1002/rm.6362>
- Quideau, S. A., McIntosh, A. C. S., Norris, C. E., Lloret, E., Swallow, M. J. B., & Hannam, K. (2016). Extraction and analysis of microbial phospholipid fatty acids in soils. *Journal of Visualized Experiments*, 114, 1–9. <https://doi.org/10.3791/54360>
- R Core Team (2019). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL <http://www.rstudio.com/>.
- Roller, B. R. K., & Schmidt, T. M. (2015). The physiology and ecological implications of efficient growth. *ISME Journal*, 9(7), 1481–1487. <https://doi.org/10.1038/ismej.2014.235>
- Rousk, J., & Bååth, E. (2011). Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiology Ecology*, 78, 17–30. <https://doi.org/10.1111/j.1574-6941.2011.01106.x>
- Rumpel, C., & Kögel-Knabner, I. (2011). Deep soil organic matter – a key but poorly understood component of terrestrial C cycle. *Plant and Soil*, 338(1), 143–158. <https://doi.org/10.1007/s11104-010-0391-5>
- Russell, J. B., & Cook, G. M. (1995). Energetics of bacterial growth: Balance of anabolic and catabolic reactions. *Microbiological Reviews*, 59(1), 48–62. <https://doi.org/10.1128/mmbr.59.1.48-62.1995>
- Ruttimann, C., Vicuna, R., Mozuch, M. D., & Kirk, T. K. (1991). Limited bacterial mineralization of fungal degradation intermediates from synthetic lignin. *Applied and Environmental Microbiology*, 57(12), 3652–3655.
- Sierra, C. A. (2012). Temperature sensitivity of organic matter decomposition in the Arrhenius equation: Some theoretical considerations. *Biogeochemistry*, 108, 1–15. <https://doi.org/10.1007/S10533-01>
- Sinsabaugh, R. L., Hill, B. H., & Follstad Shah, J. J. (2009). Eoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature*, 462(7274), 795–798. <https://doi.org/10.1038/nature08632>
- Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., & Richter, A. (2013). Carbon use efficiency of microbial communities: Stoichiometry, methodology and modelling. *Ecology Letters*, 16(7), 930–939. <https://doi.org/10.1111/ele.12113>
- Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006). Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal*, 70(2), 555–569. <https://doi.org/10.2136/sssaj2004.0347>
- Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., & Adani, F. (2010). Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. *Chemosphere*, 81(5), 577–583. <https://doi.org/10.1016/j.chemosphere.2010.08.034>
- Tambone, F., Genevini, P., D'Imporzano, G., & Adani, F. (2009). Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. *Bioresource Technology*, 100(12), 3140–3142. <https://doi.org/10.1016/j.biortech.2009.02.012>
- Tiemann, L. K., & Billings, S. A. (2011). Changes in variability of soil moisture alter microbial community C and N resource use. *Soil Biology and Biochemistry*, 43(9), 1837–1847. <https://doi.org/10.1016/j.soilbio.2011.04.020>
- Vance, G. F., & David, M. B. (1989). Effect of acid treatment on the leachate chemistry of a New England spodosol: Importance of the B horizon on dissolved organic carbon retention. *Soil Science Society of America Journal*, 53, 1242–1247. <https://doi.org/10.2134/jeq1989.00472425001800020014x>
- Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, 19(6), 703–707. [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6)
- Walsh, J. J., Rousk, J., Edwards-Jones, G., Jones, D. L., & Williams, A. P. (2012). Fungal and bacterial growth following the application of slurry and anaerobic digestate of livestock manure to temperate pasture soils. *Biology and Fertility of Soils*, 48(8), 889–897. <https://doi.org/10.1007/s00374-012-0681-6>
- Wang, G., & Post, W. M. (2012). A theoretical reassessment of microbial maintenance and implications for microbial ecology modeling. *FEMS Microbiology Ecology*, 81(3), 610–617. <https://doi.org/10.1111/j.1574-6941.2012.01389.x>
- Wang, G., Post, W. M., & Mayes, M. A. (2013). Development of microbial-enzyme-mediated decomposition model parameters through steady-state and dynamic analyses. *Ecological Applications*, 23(1), 255–272. <https://doi.org/10.1890/12-0681.1>
- Winogradzky, S. (1924). Sur la microflore autochtone de la terre arable. *Compte Rendu de l'Academie Des Sciences (Paris)*, 178, 236–239.
- WRAP (2016). DC-Agri; field experiments for quality digestate and compost in agriculture. WP1 report Appendices (Prepared by Bhogal et al.).

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