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Effects of carbon input quality and timing on soil microbe mediated processes

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ABSTRACT

Compost application is viewed as an eco-friendly and sustainable method to improve agricultural soil fertility. However, composts vary in lability and recalcitrance, which drives energy supply for microbial biomass formation and microbial processes such as soil respiration, nitrogen mineralization, and enzyme production. To provide further insights into the issue, this study aimed to elucidate how microbial activities are affected by substrate quality, amendment volume, and frequency. We proposed three hypotheses: i) microbial biomass and microbial activities increase as soon as C substrate is amended compared to control soil; ii) microbe-mediated processes are affected by substrate lability as incubation progresses; iii) repeated amendment of smaller split C source sustains a more gradual increase of microbial activities as compared to a single input. To test these hypotheses, we incubated an agricultural soil at 25 °C, adding substrates of various assimilability (glucose, cellulose and lignin) once or in 2–3 split additions. Soil respiration was determined every 2–3 days throughout incubation, while microbial biomass (MBC, MBN), inorganic N (NO_3-N and NH_4-N) concentration, and potential enzyme activities were assayed at day 4, 32, 82, and 186 following substrate amendment. Regardless of C source amendments, soil respiration increased twofold in amended soils while microbial biomass C (MBC) was 1.5 times higher compared to control soil, indicating that microbial growth was C-limited in this agricultural soil. The association between high NO_3-N concentration and low microbial biomass at the end of the incubation, regardless of amended substrate, suggested increased microbial C turnover due to C exhaustion. The addition of cellulose mostly enhanced enzyme activities of β-1,4-glucosidase (BG), β-1,4-N-acetyl glucosaminidase (NAG) and tyrosine amino peptidase (TAP) while the additions of glucose and lignin either reduced or did not affect enzyme activities compared to control at respective measuring time. Based on the result, we supposed that the selective synthesis of different enzymes by microbial community was C-source lability dependent. In conclusion, substrate quality rather than substrate amendment volume and timing had greater impacts on soil microbial activities, and hence indirectly influenced soil fertility.

1. Introduction

After decades of chemical fertilizer application, soil fertility has been severely degraded and now threatens food security and constrains efforts toward sustainable development globally (Lal, 2009). Composting is often viewed as a reliable and eco-friendly method for disposing of crop residues and improving soil quality (Hubbe et al., 2010). Composted materials originating from various categories such as wood, plant residues or manures are diverse in chemical makeup. For instances, crop residues and sludge contain high cellulosic matter content (Lalande et al., 2003) while lignin is a major component of wood (Brebu and Vasile, 2010). The most easily decomposed component, glucose, is regularly exuded from plant roots (Kuzyakov et al., 2007) and is a constituent of fresh crop residues (Shi and Marschner, 2017). Compost

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application provides soil with organic substrates of various labilities arising from their quality, defined as the availability of their C and nutrients to microbial decomposition (Niklasch and Joergensen, 2001). However, the amount of substrate applied affects its decomposition rate because decomposition increases with greater contact between substrate particles and soil colloids (Joergensen et al., 1995). In terms of substrate components, low molecular weight compounds such as glucose are readily taken up by ubiquitous soil microbial groups (Baumann et al., 2013) but the quantity of the added substance has a strong control over microbial biomass (Wu et al., 1993; Griffiths et al., 1998). Cellulose and lignin are primarily degraded by fungi (de Boer et al., 2005; Keiblinger et al., 2012; Schneider et al., 2012), but the decomposition rate also depends on microbial patterns of energy allocation (Tate, 1995) and the population dynamics of soil organic matter (SOM) degrading organisms (Fontaine et al., 2003). However, the relative influence of organic substrate quality versus the timing and volume of substrate inputs, i.e., a single large addition vs. multiple smaller additions, on microbial activity and carbon (C) turnover have not been thoroughly investigated, especially in low-C soil.

Microbial decomposers mineralize organic matter to acquire energy and nutrients, synthesize biomass, and maintain growth. Therefore, measurements of microbial biomass, C:N ratio ($MB_{C/N}$) (Powlson et al., 1987; Liu et al., 2009), and potential enzyme activities (Hoang et al., 2016) show early trends in changes of nutrient availability and C solubility resulting from agricultural practices. The variations in C pools are also reflected in basal respiration (Fang et al., 2005), which indicates C turnover and the actual mineralization rate of SOM (Borken et al., 2002). To obtain resources from complex compounds, microorganisms can synthesize extracellular enzymes with different specific functions. β-1,4-glucosidase (BG) is a cellulolytic enzyme that completes the last step of cellulose hydrolysis into glucose (German et al. 2011); β-1,4-N-acetyl glucosaminidase (NAG) is produced by fungi to cleave N-containing amino sugars from chitin (Olander and Vitousek, 2000); acid phosphatase (PHOS) can be produced by fungi and bacteria to hydrolyze organic phosphorus (P) to inorganic phosphate (Nannipieri et al., 2011; Hoang et al., 2020); and tyrosine amino peptidase (TAP) is responsible for tyrosine hydrolysis from protein and peptide substrates (Rawlings et al., 2006). The microbial strategy governing enzyme synthesis is controlled by cost:benefit ratios designed to optimally fulfill energy and nutrient demands, and is dependent on soil physical properties, nutrient availability, and competitive interactions between microbes (Allison et al., 2011).

Enzyme activities are correlated with molecular structures of the substrate (Orwin et al., 2006) or SOM turnover (Zak and Kling, 2006). Therefore, the synthesis of specific enzymes and enzyme activities are controlled by resource stoichiometry, which also mediates microbial growth and terrestrial nutrient dynamics (Zechmeister-Boltenstern et al., 2015). The ratios of C:N, C:P and N:P are determinants of ecological processes, nutrient availability, and decomposition rate of soil organic matter. However, glucose is a labile C source that can be directly taken up by soil microorganisms without the necessity of enzyme synthesis (Joergensen and Wichern, 2018). A supply of C-rich sources such as glucose, cellulose, or lignin may satisfy microbial energy demand but create unfulfilled nutrient requirements (N, P) (Hungate et al., 1997; Hamilton and Frank, 2001). As a result, microbes are induced to take up N from soil (Cheshire et al., 1999; Scheller and Joergensen, 2008), and microbes prefer to immobilize NH_4-N over NO_3-N (Bjarnason, 1987). However, the mechanisms regulating the impacts of quality and quantity of C sources on microbe mediated processes remain ambiguous.

We conducted an experiment to elucidate the interaction between the lability of C inputs, application quantity and timing (single large or split amendments) and microbial response over time. We hypothesized that i) the amendment of C compounds with various assimilabilities – fast (glucose), moderate (cellulose), and slow (lignin) – would increase soil respiration, microbial biomass, and enzyme activities compared to a control soil without substrate amendment; ii) as incubation progressed, the microbe mediated process of labile C decomposition would decline while recalcitrant substance decomposition would accelerate; iii) repeated additions of C sources to soil would sustain a more gradual increase in microbial activities as compared to a single addition. Topsoil from a potato field was incubated in the laboratory for 186 days and amended with glucose, cellulose, or lignin applied either once or split over two or three applications to provide the same total C input to all samples. Beginning at three days following C amendment, assays were conducted to quantify soil respiration, microbial biomass C and N, inorganic N (NH_4-N, NO_3-N), and potential enzyme activities.

Table 1
Experimental treatment and the associated amount of carbon addition.

	Treatment	Substrate addition on each jar, ton C ha^{-1}		
		1st day	32 d	80 d
1	Control			
2	Soil + glucose (G1)	9		
3	Soil + glucose (G2)	4.5	4.5	
4	Soil + glucose (G3)	3	3	3
5	Soil + cellulose (C1)	9		
6	Soil + cellulose (C2)	4.5	4.5	
7	Soil + cellulose (C3)	3	3	3
8	Soil + lignin (L1)	9		
9	Soil + lignin (L2)	4.5	4.5	
10	Soil + lignin (L3)	3	3	3

2. Materials and methods

2.1. Experimental design

A loamy sand topsoil (0–15 cm depth, pH: 6.1) of a potato trial field was sampled in Montcalm Research Farm, Michigan, USA (43°21′13″ N and 85 °10′33″ W). The soil comprises of 75.1% sand, 20.9% silt, 4% clay and 1.46% organic matter (Cole et al., 2020). The soil was sieved through 2 mm mesh, dried at room temperature, and homogenized. Prior to the experiment, sterilized water was added to soil to bring it to 60% of water holding capacity (WHC). Fifty grams of soil (oven-dry equivalent) was placed in each 140 mL-plastic jar to make 10 treatments and 5 replicates (Table 1). One set of soils was unamended and served as a control ("Ctrl"). Remaining soils were amended with an amount of substrate equivalent to 9 tons C ha^{-1} or 4.6 g C kg^{-1} soil, added at three different frequencies: 1) in a single addition on the first day of incubation; 2) split evenly between two applications on day 1 and 32 of incubation; and 3) split evenly between three applications on day 1, 32 and 80 of incubation. Each jar received one substrate: either glucose (powder, 40% C), cellulose (fiber, 44.4% C), or lignin (powder, 50% C; substrates obtained from Sigma-Aldrich, Germany). Soils were mixed thoroughly with substrate upon addition. This resulted in ten total treatments differing in amendment substrate and application schedule: glucose-amended soil (G1, G2, G3), cellulose amended soil (C1, C2, C3), lignin amended soil (L1, L2, L3), and one unamended control soil (Ctrl). Four sets of five replicate samples were established for each treatment, resulting in a total of 200 sample jars. Three sets of jars were allocated to destructive harvest on day 4, 34 and 82 to determine microbial biomass and activity, while the fourth set of jars was capped with rubber septa and used for determining CO_2 respiration throughout the incubation and destructive harvest on day 186. All the samples were incubated in the dark to eliminate C addition from autotrophic growth (Hernández and Hobbie, 2010) at 25 °C for 186 days.

2.2. Soil respiration

To measure CO_2 flux, incubation jars were opened for 30 min to release accumulated CO_2, then capped, and a needle and syringe were used to withdraw 1 mL of headspace gas at 0, 30 and 60 min for injection

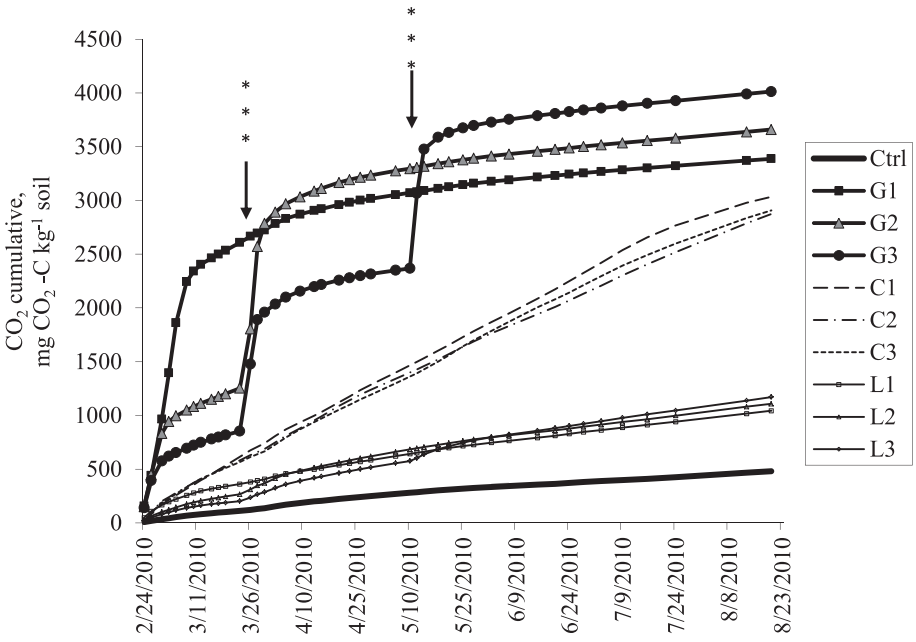


Fig. 1. Soil cumulative respiration (mg CO_2-C kg^{-1} soil). Substrate amendment boosts soil respiration (*** significant different at $p < 0.0001$). Glucose-induced respiration attains higher rate compared to cellulose and lignin effects. The pulse amendment of glucose causes a surge in soil respiration in comparison with single amendment, especially at the amendment point (demonstrated with arrows).

into an infrared gas analyzer (LICOR-820) for CO_2 measurement. The respiration rate was calculated as the average difference between CO_2 concentration at the different sampling points divided by the elapsed time. The CO_2 flux measurement was repeated every 2–5 days until the end of experiment.

2.3. Inorganic N (NO_3-N and NH_4-N)

For each sample, nitrate and ammonium were extracted from ten grams of fresh soil in 50 mL of KCl 1 M for 30 min on a shaker table. Extracts were filtered through 125 mm filter paper (Whatman #1) and stored at 4 °C to reduce ammonia volatilization and nitrate and ammonium analyses were carried out within one month after extraction. Nitrate nitrogen (NO_3^--N) concentration in the extracted solution was determined according to Doane and Horwath (2003). In the solution, vanadium reduced NO_3^- to NO_2^-, which reacted with sulfanilamide and N-1-naphthyl-ethylenediamine dihydrochloride to form a pink color that was analyzed by a spectrophotometer. Nitrate analysis was performed in 96-well clear microplates (Fisher Scientific, USA) to provide KNO_3 standard curves ranging from 0 to 10 ppm N. Four analytical replicates were run for each sample. Analytical plates were incubated at room temperature for 8–10 h before measuring nitrate concentration with a spectrophotometer at $\lambda = 540$ nm (Multiskan Ascent, Thermo Scientific, Hudson, NH). The working assay solution consisted of 25 mL of saturated vanadium solution (1 g $VaCl_3$ in 150 mL of 1 M HCl), 200 mL water, 1.65 mL of 2 % w/v sulfanilamide in 1 M HCl, and 1.65 mL of 0.2 % w/v N-(1-naphthyl)-ethylenediamine dihydrochloride in DI water, and was purged with N_2 gas to minimize vanadium oxidation during storage prior to use.

Ammonium (NH_4^+-N) was measured by the method described by Sinsabaugh et al. (2000) with some modifications in which salicylate (catalog no. 23952-66; 40 μL of dissolved reagent in deionized water) and cyanurate reagents (catalog no. 23954-66, 40 μL of dissolved reagent in deionized water) reacted with ammonium to form a green color that was measured with a spectrophotometer. Sample extracts and standard solutions were prepared as in the NO_3^--N assay. Wells containing extracts or standards each received 40 μL salicylate and 40 μL cyanurate solution prepared according to the manufacturer's instructions (Hach company, the USA). The samples were incubated in room temperature for 30 min before the NH_4^+-N concentration was determined in a microplate spectrophotometer (Multiskan Ascent, Thermo Scientific, Hudson, NH) at $\lambda = 630$ nm.

2.4. Microbial biomass C and N

Microbial biomass C and N were measured according to the chloroform fumigation/extraction method (Brookes et al., 1985; Beck et al., 1997; Robertson et al., 1999) at day five of incubation (Dilly, 2004). For each sample, fifteen g of soil at 60 % WHC was placed in a beaker in a desiccator, which also contained a beaker with 50 mL of ethanol-free chloroform and boiling chips. The desiccator was lined with wet paper towels to maintain humidity. Air in the desiccator was evacuated until the chloroform boiled for 3 min. Samples were subsequently kept in the dark at room temperature to fumigate for 24 h. The chloroform beaker was removed from the desiccator, the air was released, and a vacuum was applied six times to completely remove residual chloroform. Fumigated soil samples, as well as a set of non-fumigated control samples, were extracted with 60 mL of 0.5 M K_2SO_4 on a shaker table at 200 rpm for 1 h, then filtered (Whatman #5, 125 mm). The extracts were stored at −20 °C before analyzed for total dissolved organic C and total N with a TOC analyzer (Shimadzu, model TOC-VCPH with a TNM-1 nitrogen module attached). Microbial biomass C (MBC) was calculated as EC/kEC where EC is the chloroform-labile C pool, determined as the difference between fumigated and non-fumigated samples, and kEC is a soil-specific extraction efficiency correction factor estimated as 0.45 by Beck et al. (1997). Microbial biomass N (MBN) was calculated using the same approach but with a correction factor of 0.54 (Brookes et al., 1985).

2.5. Enzyme activities

Activities of β-1,4-glucosidase (BG), β-1,4-N-acetyl glucosaminidase (NAG), acid phosphatase (PHOS), and tyrosine amino peptidase (TAP) were determined within 24–48 h of soil sampling according to Saiya-Cork et al. (2002), Grandy et al. (2007). One g of soil and 125 mL of 50 mM sodium acetate buffer, which was pH adjusted to the average soil pH

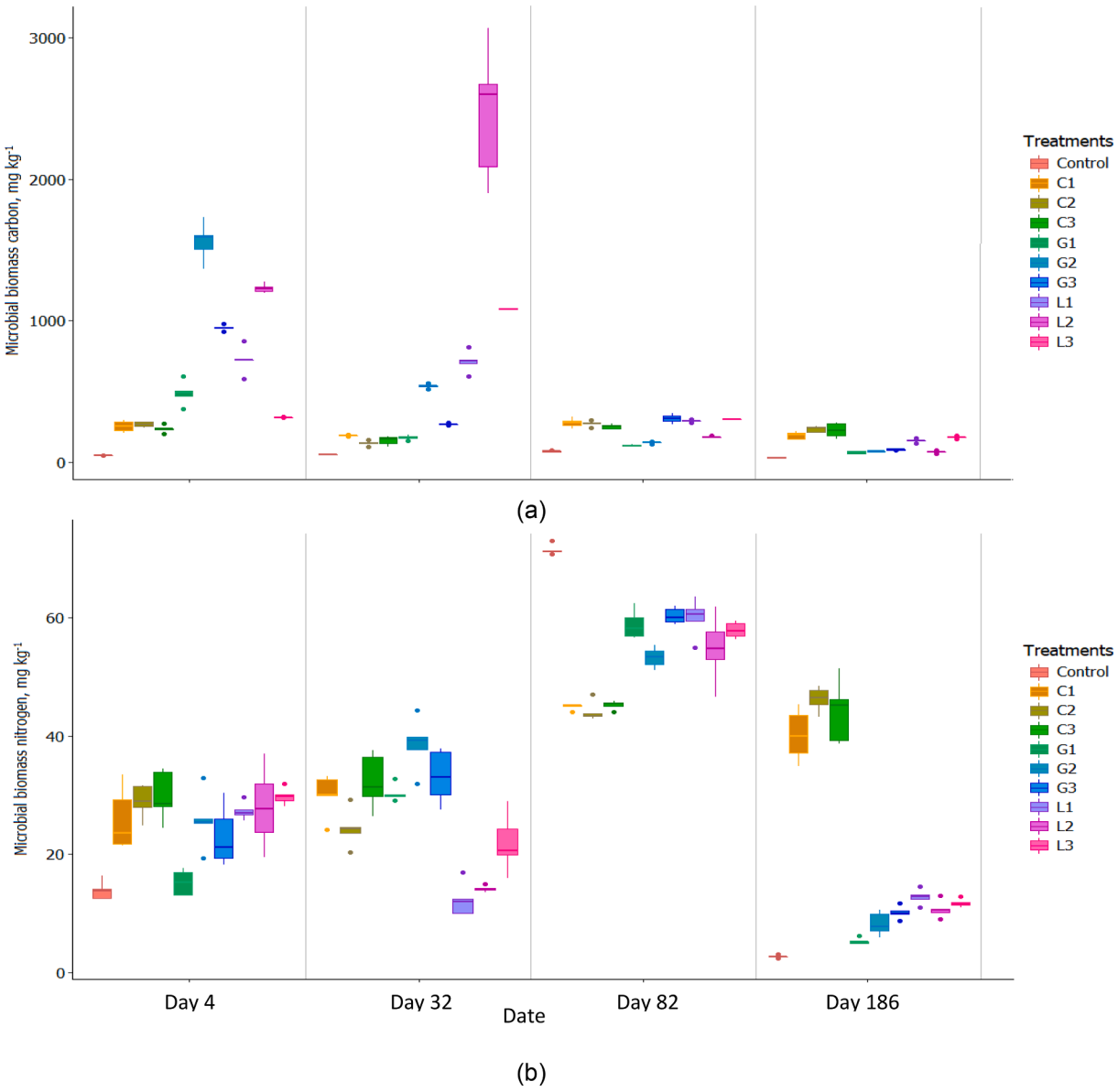


Fig. 2. Microbial biomass C (mg C kg^{-1} soil) and microbial biomass N (mg N kg^{-1} soil). Microbial biomass C (a) was boosted at least 3 times in substrate-treated samples compared to control soil regardless of measuring points. Microbial biomass N (b) reached a similar peak at day 82 as MBC but the variation is not uniformed during the experiment.

(6.5) for all the samples, were homogenized for 30 s in a commercial blender. Then, 200 µL of each soil suspension was added to a well in a black 96-well microplate (Grenier Bio-One), followed by 50 µL of substrate solution which consisted of 200 mM of 4- methylumbelliferone (MUB) conjugated to β-1,4-glucoside, β-1,4-N-acetyl glucosamine, or phosphate; or 7-amino-4-methylcoumarin (MC) conjugated to L-tyrosine (Sigma Aldrich, Germany). We included several types of control wells, each with eight replicates, to correct for background fluorescence, soil autofluorescence, and soil fluorescence quenching (Grandy et al., 2007). To be more detailed, buffer substituted for substrate solution to measure soil autofluorescence, fluorescent standard (10 mM 4-methylumbelliferone or 7-amino-4-methylcoumarin) substituted for substrate solution to measure soil fluorescence quenching, buffer substituted for soil slurry to determine background fluorescence and 50 µL of fluorescent standard, and 200 µL of buffer were used to determine the fluorescence-to-substrate-molarity conversion (Grandy et al., 2007). Plates were incubated for two hours at room temperature and reactions were terminated by adding 10 mL of 1.0 M NaOH to each well to raise the pH to 9, the optimal pH for the fluorescence of these substrates (Grandy et al., 2007). Fluorescence was quantified at 365 nm excitation with 460 nm emission filters (Fluoroskan Ascent, Thermo Scientific, Hudson, NH).

2.6. Statistical analysis

Statistical analysis was performed as a completely randomized design (RCD) analysis of variance (ANOVA) with 2 factors: 10 treatments and 4 time-points using Proc Mixed in SAS (SAS version 9.2, SAS Institute 2008). Homogeneity of variance and normality of the values were tested by Levene's test and Shapiro-Wilk's W test. We applied log transformations for NH_4^+-N and NO_3^--N data to meet assumptions of normality and equality of variance. The best model was chosen based on the AIC values for unequal variance structure models. The LSD test ($\alpha = 0.05$) was used to compare treatments across dates. Results are presented with error bars representing standard error.

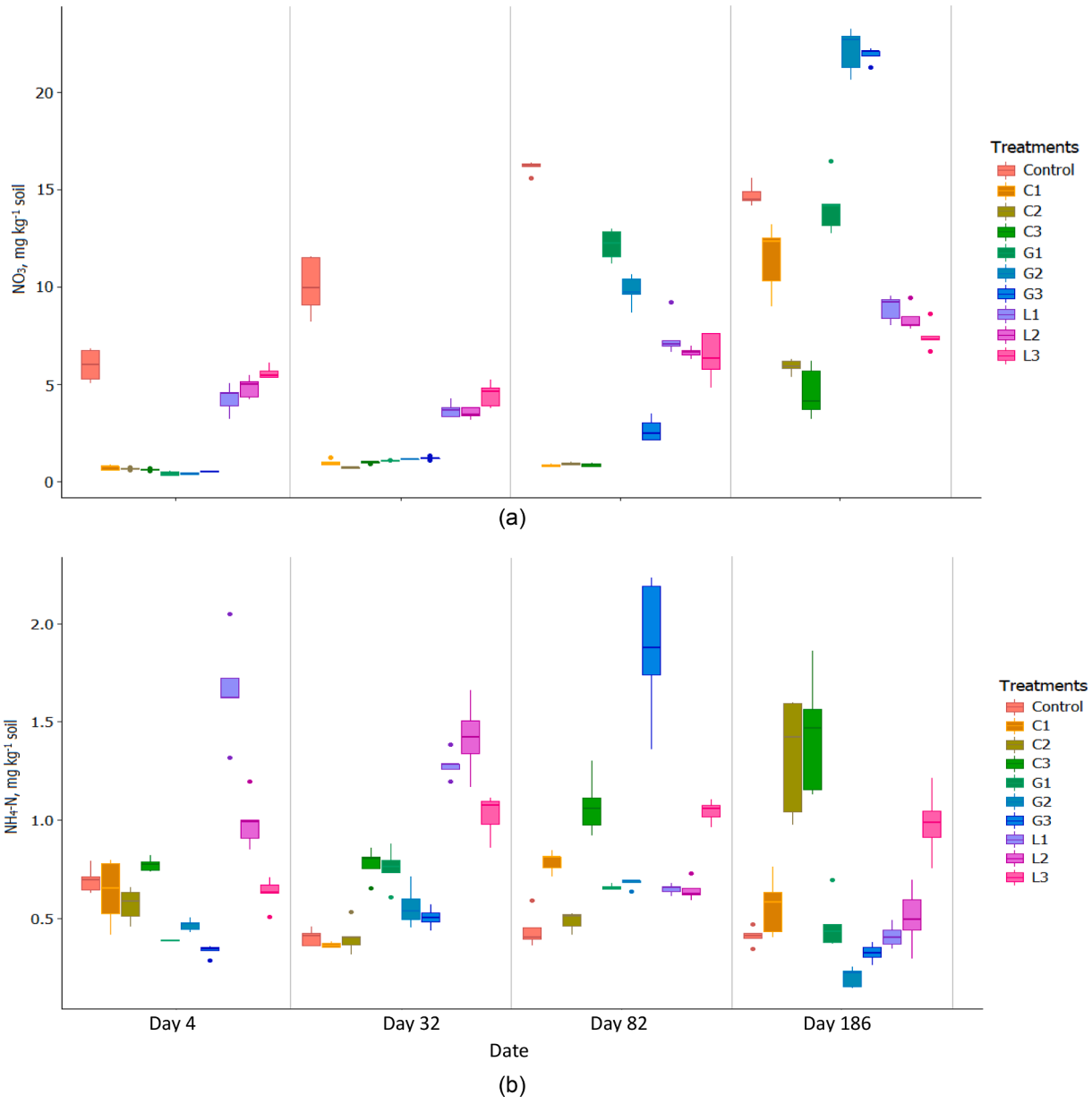


Fig. 3. Inorganic N (NO_3-N, mg kg^{-1} soil; NH_4-N, mg kg^{-1} soil). Inorganic N varies depending on substrate quality. NO_3-N in control soil is significantly higher than those in substrate-treated soil except for the last period of the experiment with the pulse application of glucose (a). NH4-N was not uniformly affected by substrate amendment but slightly increased compared to control soil (b).

3. Results

3.1. Soil respiration

Substrate amendment accelerated soil respiration relative to the control in glucose (7x), cellulose (6x) and lignin (2x) treatments by the end of the experiment (Fig. 1). Glucose amendment boosted CO_2 respiration rate by an order of magnitude at the initial measurement while the respiration rate induced by the other two substrates only increased a maximum of 6.2 times compared to the control (Appendix 1). While cumulative respiration in cellulose and lignin treatments increased linearly with time, respiration in glucose treatments followed a logistic curve (Fig. 1). Cumulative CO_2 rapidly increased ($p < 0.001$, denoted as asterisks) at the time of each glucose addition (denoted as arrows; Fig. 1). Respiration rates approached zero after approximately 120 days of glucose incubation (Supplementary Fig. 1), and total cumulative respiration tended to be highest in the treatment that received the amendment over three time points. However, dividing cellulose or lignin amendments into two or three applications did not significantly affect cumulative CO_2 production compared to a single application.

3.2. Microbial biomass

Microbial biomass C (MBC) increased at least 1.5 times in substrate-treated soil compared to control soil at every time point (Fig. 2a). Glucose and lignin-amended soils had up to 6 times of MBC higher than that of cellulose-amended soils at days 1 and 32. While MBC was relatively stable throughout the incubation in cellulose treatments, glucose and lignin treatments had sharp decreases in MBC at days 82 and 186.

MBN peaked in all treatments at day 82 (Fig. 2b), then dramatically declined in glucose and lignin treatments but remained high in cellulose treatments regardless of substrate addition timing. MBN was higher in lignin amended soils than in control soils in the measurement at days 4, 82 and 186. MBN in the control treatment was not measured on day 34.

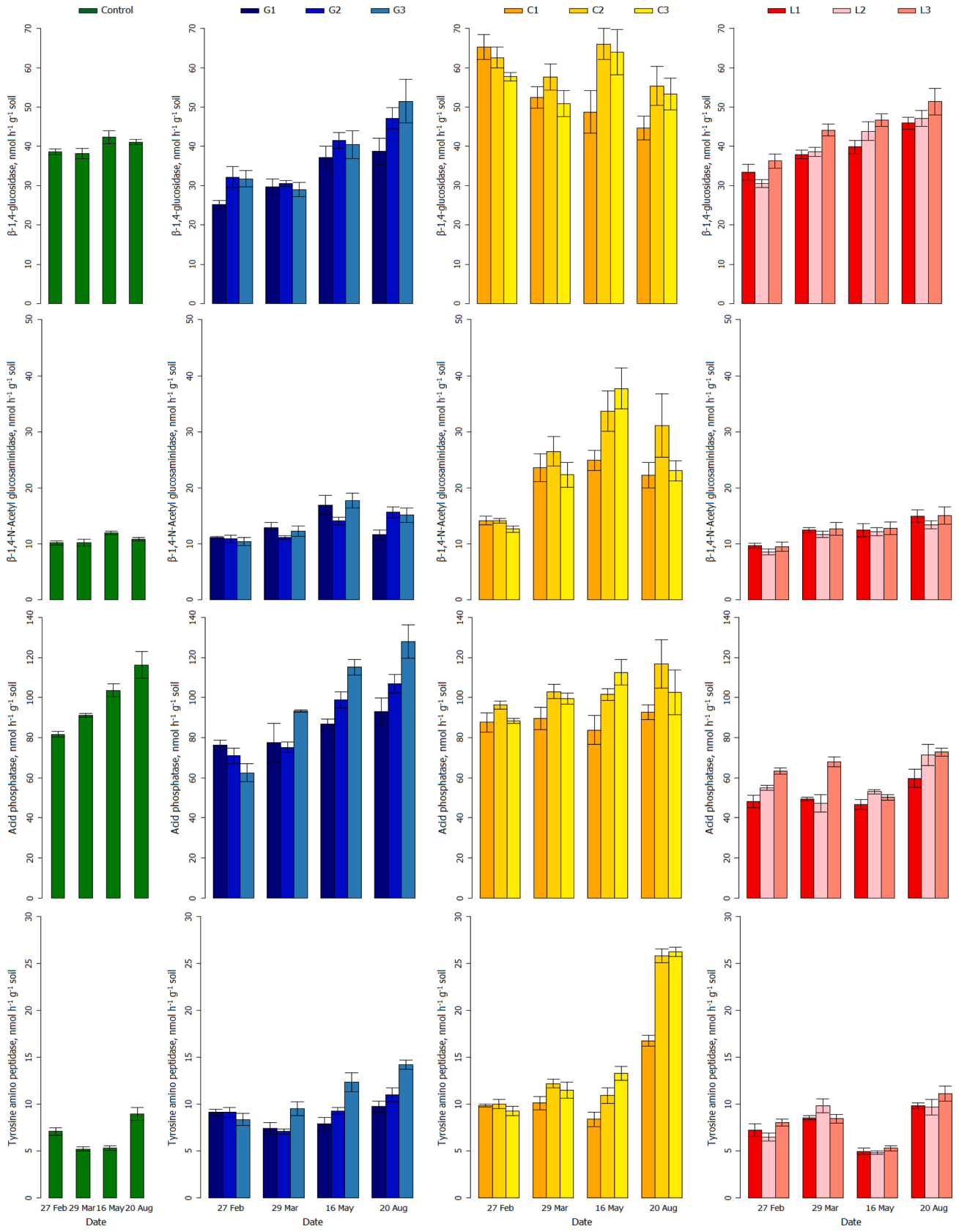


Fig. 4. Activities of β-1,4-glucosidase (BG), β-1,4-N-Acetyl glucosaminidase (NAG), acid phosphatase (PHOS) and tyrosine amino peptidase (TAP) were determined at 4 respective times. Substrate amendment demonstrated impacts on most enzymes but cellulose released significantly higher impacts than other substrates.

3.3. Inorganic nitrogen

NO_3-N in control soil was significantly higher than in substrate-treated soils, except for the split application of glucose treatments at day 186 (Fig. 3a). Generally, the application of any substance raised NO_3-N over time. Nitrate-N increased fifty-fold in split-application glucose treatments between days 4 and 186 and doubled or tripled the concentration found in cellulose or lignin treatments at the end of the experiment. Cellulose amendment only significantly increased NO_3-N concentration at the final harvest point.

NH_4-N was not uniformly affected by substrate amendment. Ammonium-N concentrations exceeded that of control soil on day 34 in soils amended with glucose in single application, and on day 82 in soils with glucose amendments split over three time points (Fig. 3b). On day 82, NH_4-N concentrations rose modestly by 13% above the control that received three cellulose amendments, while on day 34 large single lignin application treatments greatly elevated concentrations of NH_4-N up to 141% that of control soils.

3.4. Enzyme activity

Compared to control soil, potential activity of BG was 53% lower in soils that received a single addition of glucose; only in split-amendment glucose treatments at the end of the experiment did potential BG activity exceed that of the control, and only by 14% ($p < 0.05$) (Fig. 4a). Lignin amendment resulted in similarly gradual increases in potential BG activity over time but the significantly higher BG activity than control soil was only found in two and three single amendment of lignin. At all-time points, potential BG activity was greater in cellulose-treated than control soil. Over the course of the incubation, potential BG activity gradually declined in soils that received a single addition of cellulose, while the split-amendment cellulose treatments had greater potential BG activity at day 4 and 82 than at day 34 and 186. BG activities in glucose- and lignin- amended soils were negatively correlated with MBC ($p < 0.05$) while the correlation between MBC and BG was positive in cellulose-amended soils ($p < 0.05$) (Appendix 2).

From day 34 through the end of the experiment, potential NAG activity was higher with glucose and lignin amendment than in the control, but cellulose amendment boosted NAG activity beginning at day 4 by up to 200% compared to control soil and 100% compared to glucose or lignin treatments. Soil amended with cellulose had a positive correlation between MBN and NAG ($r > 0.9$, $p < 0.05$).

Potential PHOS activities in control soils increased significantly over time in this incubation. Compared to the control, potential PHOS activity was substantially lower in all lignin-amended soils, and significantly lower in soils amended singly or in two applications of glucose and cellulose at respective measuring points. Glucose amendment split over three applications led to a gradual increase of PHOS with time, which was significantly higher than that of the control from day 34 onward.

While glucose and cellulose addition significantly raised potential TAP activity compared to control soil throughout the experiment ($p < 0.05$), soils amended with lignin had TAP activity similar to that of control soils at all harvest points except day 34, when potential TAP activity was greatly increased.

4. Discussion

Three hypotheses have been invoked that i) the amendment of C compounds at various assimilable capacities, fast (glucose), moderate (cellulose) and slow (lignin), induces soil respiration and microbial biomass and enhances enzyme activities compared to control soil (without substance amendment); ii) as the substrate incubation time prolongs, the microbially mediated processes of labile C decomposition decreases in contrast to an acceleration of recalcitrant substance decomposition; iii) the continuous multiple addition of C sources to soil sustains a steady increase of microbial activities compared to a single addition.

4.1. Substrate labilities determine the volume of C turnover and microbial activities

4.1.1. CO_2 respiration induced by substrate amendment

As we hypothesized, all C inputs, regardless of assimilability or application schedule, strongly stimulated respiration flux compared to controls (Fig. 1), implying that the microbial community is C-limited in this agriculture soil (Bowen & Rovira, 1991; Darrah, 1995; Bandopadhyay et al., 2018). Most soil microorganisms are in a dormant state under field conditions of restricted C sources (Anderson and Domsch, 1985; Joergensen and Wichern, 2018), so they quickly respond to C inputs, as illustrated by the elevation in CO_2 at the day 3 of our experiment (Appendix 1). Unfortunately, our experimental design did not allow us to partition the contributions of C inputs versus native SOM decomposition (priming effects) to elevated CO_2. Of the three C sources we used as amendments, glucose is the most easily assimilable substance and thus induces faster and larger increases in soil respiration than cellulose or lignin does. Our results are in line with previous findings that sugar is a major substrate utilized by microorganisms in the early stages of composting (Xie et al., 2014). The low solubility of cellulose and lignin could result in the lower accessibility of microbes to these amended substrates (Schutter and Dick, 2001), consistent with our observation of retarded degradation of cellulose and lignin compared to glucose. Alternatively, slow-growing fungi responsible for cellulose and lignin decomposition could have been outcompeted by the fast-growing microbes that decompose sugar (Coûteaux et al., 1991). A lag time in the decomposition of cellulose and lignin could represent the additional time required for the soil microbial community to adapt to these more complex substrates (Schutter and Dick, 2001) as shown in the gradual mineralization of cellulose and lignin early in our incubation (Torres et al., 2014). Compared to cellulose, lignin has a more complex molecular structure (Kögel Knabner, 2002) and is decomposed by a more limited number of specialized soil bacteria and fungi (Berg et al., 2008). The properties of lignin could explain why C turnover with lignin amendment was less than C turnover with cellulose amendment.

The removal of C limitation from this agriculture soil is responsible for the prompt increase of CO_2 respiration after substrate amendment. The stimulation of different microbial groups by different substrate labilities plausibly explains the distinction in substrate decomposition rate expressed by C turnover.

4.1.2. Short-term increase microbial biomass after substrate amendment

In addition to increasing respiration, substrate amendment also enhanced microbial biomass. This implies that all amended substrates irrespective of their labilities provided relatively sufficient C to enable microbial growth in this experiment (Reischke et al., 2015). However, MBN fluctuations in amended soils compared to controls could demonstrate that N also constrains microbial utilization of added substrates. For each type of substrate amendment, MBC peaked simultaneously with soil respiration rate (Supplementary 1). The finding aligns with previously observed correlations between microbial biomass and substrate respiration (Waldrop and Firestone, 2004; Brant et al., 2006). However, the fact that MBC peaked in lignin-amended soils later than in glucose- and cellulose-amended soils suggests that microorganisms need more time to adapt to lignin amendment than those of glucose or cellulose. Soil MBC increased more slowly in cellulose than in glucose or lignin treatments during the first half of the incubation, perhaps because microbial assimilation of C from pure cellulose is inefficient. A low C use efficiency of cellulose is also illustrated by a relatively stable MBC throughout the experiment in this treatment. Our hypothesis is supported by Miao et al. (2021) who attributed higher abundance of fast-growing bacteria (Gram-negative, G-) in compost soil to lower carbon use efficiency in response to cellulose. The shift of microbial

communities with different substrate preference and growth strategies mediated the decomposition and accumulation of cellulose in soil (Herrmann et al., 2014; Riggs and Hobbie, 2016; Miao et al., 2021). Unlike cellulose, lignin is only decomposed by selective microbial groups with higher C assimilation efficiencies (DeAngelis et al., 2011; Torres et al., 2014). These specialized lignin-decomposers may be responsible for the large microbial biomass C pool observed in the lignin treatment during the initial incubation period. The microbial biomass C:N ratio ($MB_{C/N}$) is significantly higher in lignin-amended soil than that of control soil in all but the last measurement (Appendix 3). The result further suggests that this C input shifted the microbial community composition since fungal taxa have higher biomass C:N ratios than bacteria (bacteria C:N ratio ~ 5:1, fungi C:N ratio ~ 15:1; Pichtel and Hayes, 1990; Joergensen et al., 1995; Sterner and Elser, 2002).

In short, the amended substrates met an energy threshold that results in the short-term increase of MBC after substrate amendment. Selective stimulation of different microbial communities by respective substrate labilities explains the distinction in MBC peaks during the incubation.

4.1.3. Long-term decrease of microbial biomass due to soil nutrient limitation

Differential responses of microbial taxa to substrate lability corroborate some of the MBC dynamics we observed. Early in the incubation, glucose amendment may have mainly activated fast-growing r-strategists (Blagodatskaya et al., 2009) whose quick growth initiated the decomposition of SOM to fulfil their demand for N (Swift et al., 1979). A dramatic decrease in MBC after 34 days of incubation in glucose-amended soils can be explained by the return to dormancy of fast-growing r-strategists as glucose-C is exhausted (Wu et al., 1993). Microbial C assimilation requires the availability of additional nutrients to fulfill all stoichiometric requirements so the amendment of C sources without nutrient supplies would hamper microbial growth, as we observed in the drastic decline in microbial biomass after 30 days of lignin and glucose incubation. During the incubation, microbial biomass C production induced by cellulose was relatively stable (Fig. 2). This could be resulted from less efficient microbial community (Miao et al., 2021) as we mentioned above.

The supply of N-free substrates causes subsequent stress on N demand for C assimilation that induces a dramatic reduction of MBC during the incubation. Apparently, the lack of N sources triggers C storage preference rather than microbial growth with prolonged incubation time (Nguyen and Guckert, 2001; Torres et al., 2014).

4.1.4. Stimulation of enzyme activities depend on substrate lability and microbial strategy for nutrient or energy acquisition

The addition of cellulose induced a greater increase of BG and NAG activities than lignin or glucose amendments did (Fig. 4). The result again suggests that this soil was C-limited. β-1,4-glucosidase (BG) is responsible for cellobiose degradation to glucose, so glucose addition hampers the synthesis of BG due to "evolutionary-economic" principles (Allison et al., 2011). According to this principle, to favor microbial growth, microorganisms will minimize metabolic costs by reducing enzyme production whenever microbial demands can be met by available C and nutrients. Enzyme synthesis costs both energy and N (Allison and Vitousek, 2005), but due to the stable structure of cellulose, its amendment to soil may shift enzyme activity towards N-pool decomposition due to cellulose's relatively stable structure (Wutzler et al., 2017). Glucose provides readily available C for assimilation so microbes can invest their energy to acquire other nutrients needed for efficient microbial growth. The increase of BG and NAG in glucose-amended soils toward the end of the experiment could have occurred as the labile C in glucose was exhausted and microbes were forced to produce enzymes for metabolism of alternate substrates. The addition of glucose and lignin leads to lower enzyme activities, but higher MBC production compared to cellulose, suggesting that C assimilated from glucose and lignin is invested more efficiently in biomass instead of enzyme production. A positive relationship between the activity of more labile C-acquiring enzymes and soil respiration was reported by Hernández and Hobbie (2010) but they assumed that total enzyme activities rather than specific enzyme activity are responsible for this correlation.

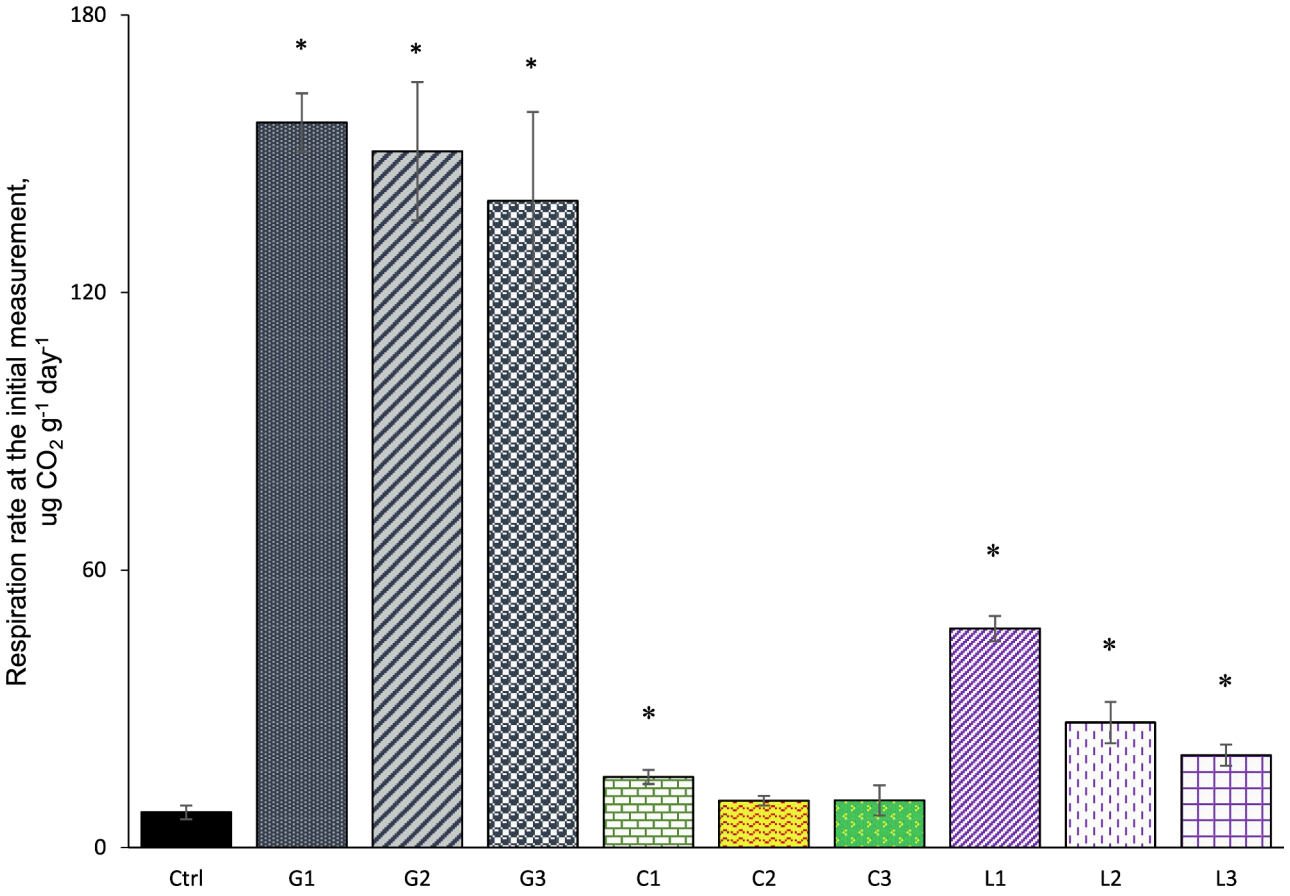
Glucose and cellulose amendments did not significantly influence potential PHOS activity, but lignin additions significantly lowered the activity of PHOS ($p < 0.05$). These findings have shown that either i) lignin suppressed microbial synthesis of PHOS or ii) lignin induced a shift of microbial groups to those with demands for nutrients other than P. Tyrosine amino peptidase (TYR) is an enzyme catalyzing the decomposition of phenolic compounds originated from plants and microorganisms. Meanwhile, lignin is an aromatic component of humic substances (Gerke, 2018) so the increase of lignin may slightly inhibit TYR activity (Ruggiero and Radogna, 1988). However, we could not definitely confirm this hypothesis in this experiment.

4.2. Greater increase of microbial activities in split applications of C sources than single addition

Our substrate additions, when divided over two to three applications, were equivalent to an intermediate amendment concentration (1.4–3.6 mg C g^{-1} soil) as suggested by De Graaff et al. (2010). Although the study provided the same total substrate amendment as the single-amendment treatment, the divided amendments induced greater cumulative respiration, particularly in the case of easily-assimilable glucose. This finding supports our third hypothesis that microbial activity increases more with recurring, smaller additions of C sources than with a single large addition. It is possible that the first small amendment of glucose not only provided as C source for active microorganisms, but also activated dormant microbial groups, which would in turn increase overall microbial C requirements (Kuzyakov et al., 2007; De Graaff et al., 2010). However, split inputs of glucose are quickly assimilated for biomass growth and can result in intermittent microbial growth and C exhaustion (Kuzyakov et al., 2007; De Graaff et al., 2010).

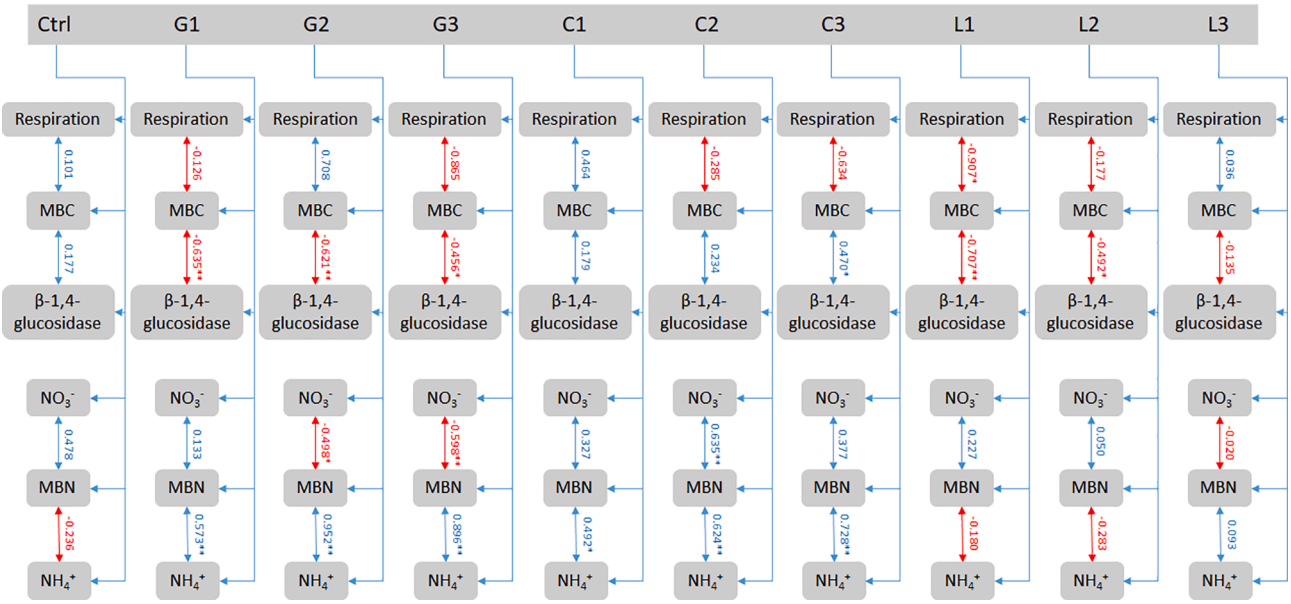
Our observation shows that multiple smaller additions of glucose increased MBC more than a large single amendment. This result means that either i) divided substrate applications may have increased C use efficiency (Vinolas et al., 2001) or stimulated microbial species with higher growth efficiency and therefore increased biomass production (Xu et al., 2014; Bonner et al., 2018); or ii) single large substrate additions may have reduced C use efficiency. In contrast, cellulose and lignin are more recalcitrant substances, so microbial communities need much more time to adapt and degrade these two substrates regardless of whether additions are single or split. Therefore, substrate recalcitrance could eliminate the impact of split additions on substrate turnover and other microbial activities. Although the addition of C sources impacted soil N availability in this incubation, we did not observe a consistent effect of amendment timing treatment on N turnover. The increase in N availability following C amendment in our study may indicate that soil microbes had selective demand for N.

There is no single explanation for the impacts of single or split C additions on enzyme activities (Hernández and Hobbie, 2010). B-1,4-glucosidase (BG) is a mediator of cellulose decomposition (Sinsabaugh and Linkins, 1988), so the increase of BG activity resulting from cellulose amendment is correlated with soil carrying capacity in which microbial biomass and enzyme activities are maintained at steady state condition by microbial communities (Shackle et al., 2000). Although MBC remained low and stable after cellulose amendment, increased potential BG activity in this treatment suggests a low carbon efficiency of microbial decomposers. Other studies have noted a suppressive effect of lignin on enzyme activities and concluded that highly polyphenolic compounds bind with and inactivate enzymes. The findings support the reduction of most of enzyme activity in our lignin-amended soils compared to control soils (Kraus et al. 2003; Allison, 2006). A significant increase of NAG activities over time, especially in the cellulose treatment, apparently explains the slight increase of NH_4-N concentration

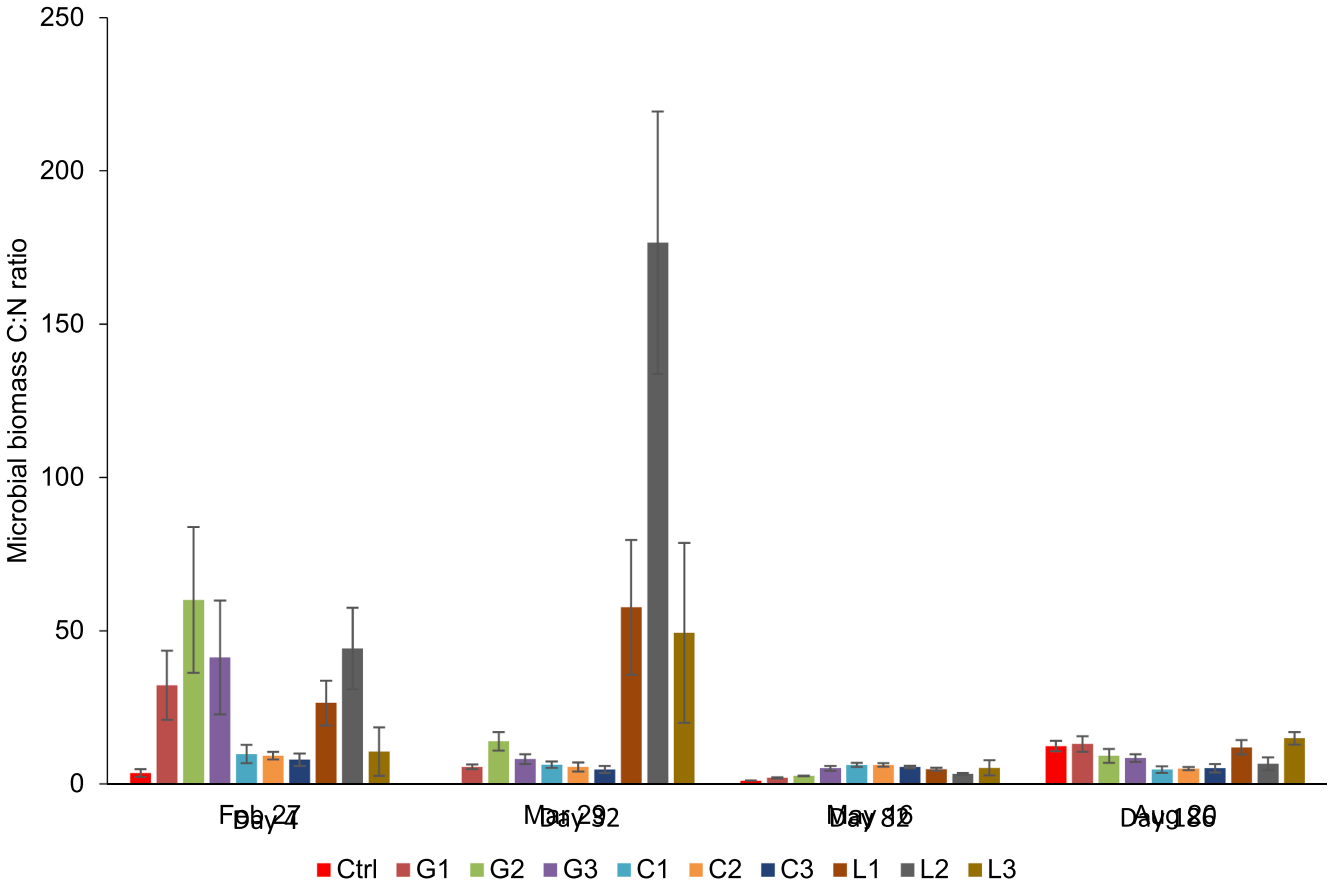


Appendix 1. Glucose amendment boosted CO_2 respiration rate 18 times at the initial measurement while the respiration rate induced by the other two substrates only increased maximum 6.2 times in comparison with control. The asterisk "*" indicated a significant difference from control soil.

and, hence, MBN value. The acceleration or depression of enzyme synthesis under different substrate amendments has been associated with nutrient availability (Chróst, 1991; Shackle, 2000) or energy demands of decomposer groups (Sinsabaugh et al., 1993). Therefore, the balance between C inputs and outputs via microbial respiration determines the SOM decomposition rate (De Graaff et al., 2010) but the volume and timing of substrate supply plays a pivotal role in driving microbial activities and subsequent decomposition processes.



Appendix 2. Correlation between respiration vs. MBC, MBC vs, BG vs. MBC, inorganic N vs. MBN. The green numbers showed a positive correlation and the red numbers demonstrated a negative correlation ("*": $p < 0.05$; "**": $p < 0.01$).



Appendix 3. Microbial biomass C:N ratio were higher in glucose and lignin amendments than control soil implied a dominant of fungi over bacteria in soil. The reduction of C:N ratio with time in cases of glucose and lignin amendments proved a shift in microbial community composition during substrate decomposition.

5. Conclusion

We found that the lability of amended substrates had greater effects on microbial activities than amendment amount and frequency. Regardless of their assimilability, supplying C sources triggered microbial respiration in incubated soils, especially soon after amendment, which implied a lack of energy sources for the decomposer community. However, substrates of different labilities selectively triggered different microbial activities with important consequences for substrate mineralization and subsequent SOM decomposition. A short-term increase of microbial biomass after substrate amendment was followed by a long-term decrease mainly due to N limitation. Increases in respiration, microbial biomass, and enzyme activity with split compared to single amendment were only found with glucose, likely due to the greater recalcitrance of lignin and cellulose. This experiment sheds light on not only the importance of substrate lability but also on the amount and timing of substrate applications during soil amendment. The results should be considered when applying slower-release fertilizers like compost or organic manure to agricultural soil instead of immediately available chemical fertilizers to improve soil fertility.

Declaration of Competing Interest

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

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Appendix A

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115605.

References

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrientinputs. Soil Biol. Biochem. 37 (5), 937–944. https://doi.org/10.1016/j.soilbio.2004.09.014.

Allison, S.D., 2006. Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. Biogeochemistry 81 (3), 361–373.

Allison, S.D., Weintraub, M.N., Gartner, T.B., Waldrop, M.P., 2011. Evolutionary economic principles as regulators of soil enzyme production and ecosystem function. In: Shukla, G., Varma, A. (Eds.), Soil Enzymology. Springer-Verlag, Berlin, Germany, pp. 229–243.

Anderson, T.-H., Domsch, K.H., 1985. Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biol. Fertil. Soils 1, 81–89.

Bandopadhyay, S., Martin-Closas, L., Pelacho, A.M., DeBruyn, J.M., 2018. Biodegradable plastic mulch films: impacts on soil microbial communities and ecosystem functions. Front. Microbiol. 9, 819. https://doi.org/10.3389/fmicb.2018.00819.

Baumann, K., Dignac, M.-F., Rumpel, C., Bardoux, G., Sarr, A., Steffens, M., Maron, P.-A., 2013. Soil microbial diversity affects soil organic matter decomposition in a silty grassland soil. Biogeochemistry 114 (1-3), 201–212.

Beck, T., Joergensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R., Scheu, S., 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol. Biochem. 29 (7), 1023–1032.

Berg B, McClaugherty C (eds.), 2008. Plant Litter. Decomposition, Humus Formation, Carbon Sequestration. Springer, Berlin.
Bjarnason, S., 1987. Immobilization and remineralization of ammonium and nitrate after addition of different energy sources to soil. Plant Soil 97 (3), 381–389.
Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.-H., Kuzyakov, Y., 2009. Contrasting effects of glucose, living roots and maize straw on microbial growth kinetics and substrate availability in soil. Eur. J. Soil Sci. 60, 186–197.
Bonner, M.T.L., Shoo, L.P., Brackin, R., Schmidt, S., 2018. Relationship between microbial composition and substrate use efficiency in a tropical soil. Geoderma 315, 96–103.
Borken, W., Xu, Y.-J., Davidson, E.A., Beese, F., 2002. Site and temporal variation of soil respiration in European beech, Norway spruce, and Scot pine forest. Global Change Biol. 8, 1205–1216.
Bowen, G.D., Rovira, A.D., 1991. The rhizosphere, the hidden half of the hidden half. In: Waisel, Y., Eshel, A., Kafkafi, U. (Eds.), Plant Roots: The Hidden Half. Marcel Dekker Inc., New York, pp. 641–649.
Brant, J.B., Sulzman, E.W., Myrold, D.D., 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biol Biochem 38 (8), 2219–2232.
Brebu, M., Vasile, C., 2010. Thermal degradation of lignin – a review. CellulChem Technol. 44, 353–363.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842.
Cheshire, M.V., Bedrock, C.N., Williams, B.L., Chapman, S.J., Solntseva, I., Thomsen, I., 1999. The immobilization of nitrogen by straw decomposing in soil. Eur. J. Soil Sci. 50, 329–341.
Chróst, R.J., 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: Chróst, R.J. (Ed.). Microbial Enzymes in Aquatic Environments, Springer, New York, 29–59.
Cole, E., Pu, J., Chung, H., Quintanilla, M., 2020. Impacts of manures and manure-based composts on root lesion nematodes and *Verticillium dahliae* in Michigan potatoes. Phytopathology 110. https://doi.org/10.1094/PHYTO-11-19-0419-R.
Coûteaux, M.-M., Mousseau, M., Célérier, M.-L., 1991. Increased atmospheric CO_2 and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. Oikos 61, 54–64.
Darrah, P.R., 1995. Rhizodeposition under ambient and elevated CO2 levels. Plant Soil 187 (2), 265–275.
de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol. Rev. 29 (4), 795–811.
de Graaff, M., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytol. 188 (4), 1055–1064.
DeAngelis, K.M., Allgaier, M., Chavarria, Y., Fortney, J.L., Hugenholtz, P., Simmons, B., Sublette, K., Silver, W.L., Hazen, T.C., Rao, C.V., 2011. Characterization of trapped lignin-degrading microbes in tropical forest soil. PLoS ONE 6 (4), e19306. https://doi.org/10.1371/journal.pone.0019306.
Dilly, O., 2004. Effects of glucose, cellulose, and humic acids on soil microbial eco-physiology. J. Plant Nutr. Soil Sci. 167 (3), 261–266.
Doane, T.A., Horwáth, W.R., 2003. Spectrophotometric determination of nitrate with a single reagent. Anal. Lett. 36 (12), 2713–2722.
Fang, C., Smith, P., Moncrieff, J.B., Smith, J.U., 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature 433 (7021), 57–59.
Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biol Biochem 35 (6), 837–843.
Gerke, J., 2018. Concepts and misconceptions of humic substances as the stable part of soil organic matter: a review. Agronomy 8 (5), 76. https://doi.org/10.3390/agronomy8050076.
German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol. Biochem. 43 (7), 1387–1397.
Grandy, A.S., Neff, J.C., Weintraub, M.N., 2007. Carbon structure and enzyme activities in alpine and forest ecosystems. Soil Biol. Biochem. 39 (11), 2701–2711.
Griffiths, B.S., Ritz, K., Ebblewhite, N., Dobson, G., 1998. Soil microbial community structure: effects of substrate loading rates. Soil Biol. Biochem. 31 (1), 145–153.
Hamilton, E.W., Frank, D.A., 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82 (9), 2397–2402.
Hernández, D.L., Hobbie, S.E., 2010. The effects of substrate composition, quantity, and diversity on microbial activity. Plant Soil 335 (1-2), 397–411.
Herrmann, A.M., Coucheney, E., Nunan, N., 2014. Isothermal microcalorimetry provides new insight into terrestrial carbon cycling. Environ. Sci. Technol. 48, 4344–4352.
Thu Hoang, D.T., Maranguit, D., Kuzyakov, Y., Razavi, B.S., 2020. Accelerated microbial activity, turnover and efficiency in the drilosphere is depth dependent. Soil Biol. Biochem. 147, 107852. https://doi.org/10.1016/j.soilbio.2020.107852.
Hoang, D.T.T., Razavi, B.S., Kuzyakov, Y., Blagodatskaya, E., 2016. Earthworm burrows: kinetics and spatial distribution of enzymes of C-, N- and P- cycles. Soil Biol. Biochem. 99, 94–103.
Hubbe, M., Nazhad, M., Sanchez, C., 2010. Cellulosic biomass and organic waste into high-value soil amendments: a review. BioResources 5 (4), 2808–2854.
Hungate, B.A., Chapin III, F.S., Zhong, H., Holland, E.A., Field, C.B., 1997. Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. Oecologia 109 (1), 149–153.
Joergensen, R.G., Schmaedeke, F., Windhorst, K., Meyer, B., 1995. Biomass and activity of microorganisms in a fuel oil contaminated soil. Soil Biol. Biochem. 27 (9), 1137–1143.
Joergensen, R.G., Wichern, F., 2018. Alive and kicking: why dormant soil microorganisms matter. Soil Biol. Biochem. 116, 419–430.
Keiblinger, K.M., Schneider, T., Roschitzki, B., Schmid, E., Eberl, L., Hämmerle, I., Leitner, S., Richter, A., Wanek, W., Riedel, K., Zechmeister-Boltenstern, S., 2012. Effects of stoichiometry and temperature perturbations on beech leaf litter decomposition, enzyme activities and protein expression. Biogeosciences 9 (11), 4537–4551.
Kraus, T.E.C., Dahlgren, R.A., Zasoski, R.J., 2003. Tannins in nutrient dynamics of forest ecosystems – a review. Plant Soil 256 (1), 41–66.
Kuzyakov, Y., Hill, P.W., Jones, D.L., 2007. Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. Plant Soil 290 (1-2), 293–305.
Kögel Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs into soil organic matter. Soil Biol. Biochem. 34, 139–162.
Lal, R., 2009. Challenges and opportunities in soil organic matter research. Eur. J. Soil Sci. 60, 158–169.
Lalande, R., Gagnon, B., Simard, R.R., 2003. Papermill biosolid and hog manure compost affect short-term biological activity and crop yield of a sandy soil. Can. J. Soil Sci. 83 (4), 353–362.
Liu, M., Hu, F., Chen, X., Huang, Q., Jiao, J., Zhang, B., Li, H., 2009. Organic amendments with reduced chemical fertilizer promote soil microbial development and nutrient availability in a subtropical paddy field. The influence of quantity, type and application time of organic amendments. Appl. Soil Ecol. 42 (2), 166–175.
Miao, Y., Niu, Y., Luo, R., Li, Y., Zheng, H., Kuzyakov, Y., Chen, Z., Liu, D., Ding, W., 2021. Lower microbial carbon use effciency reduces cellulose-derived carbon retention in soils amended with compost versus mineral fertilizers. Soil Biol. Biochem. 156, 108227.
Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of phosphatase enzymes in soil. In: Bunemann, E.K., Obreson, A., Frossard, E. (Eds.), Phosphorus in Action. Springer, Berlin, pp. 215–243.
Nguyen, C., Guckert, A., 2001. Short-term utilisation of C-14-[U] glucose by soil microorganisms in relation to carbon availability. Soil Biol. Biochem. 33, 53–60.
Niklasch, H., Joergensen, R.G., 2001. Decomposition of peat, biogenic municipal waste compost and shrub/grass compost added in different rates to a silt loam. J. Plant Nutr. Soil Sci. 164, 365–369.
Olander, L.P., Vitousek, P.M., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49, 175–190.
Orwin, K.H., Wardle, D.A., Greenfield, L.G., 2006. Ecological consequences of carbon substrate identity and diversity. Ecology 87, 580–593.
Pichtel, J.R., Hayes, J.M., 1990. Influence of fly ash on soil microbial activity and populations. J. Environ. Qual. 19 (3), 593–597.
Powlson, D.S., Prookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biol. Biochem. 19 (2), 159–164.
Rawlings, N.D., Morton, F.R., Barrett, A.T., 2006. MEROPS: the peptidase database. Nucl. Acids Res 32, D160–D164.
Reischke, S., Kumar, M.G.K., Bååth, E., 2015. Threshold concentration of glucose for bacterial growth in soil. Soil Biol. Biochem. 80, 218–223.
Riggs, C.E., Hobbie, S.E., 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biol. Biochem. 99, 54–65.
Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P., 1999. Standard soil methods for long-term ecological research. Oxford University Press, Oxford, UK.
Ruggiero, P., Radogna, V.M., 1988. Humic acids-tyrosinase interactions as a model of soil humic-enzyme complexes. Soil. Biol. Biochem. 20 (3), 353–359.
Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biol. Biochem. 34 (9), 1309–1315.
Scheller, E., Joergensen, R.G., 2008. Decomposition of wheat straw differing in nitrogen content in soils under conventional and organic farming management. J. Plant Nutr. Soil Sci. 171 (6), 886–892.
Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., Riedel, K., 2012. Who is who in litter decomposition? Metaproteomics reveals majormicrobial players and their biogeochemical functions. ISME J. 6 (9), 1749–1762.
Schutter, M., Dick, R., 2001. Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. Soil Biol. Biochem. 33 (11), 1481–1491.
Shackle, V.J., Freeman, C., Reynolds, B., 2000. Carbon supply and the regulation of enzyme activity in constructed wetlands. Soil Biol. Biochem. 32 (13), 1935–1940.
Shi, A., Marschner, P., 2017. Soil respiration and microbial biomass in multiple drying and rewetting cycles – effect of glucose addition. Geoderma 305, 219–227.
Sinsabaugh, R.L., Antibus, R.K., Linkins, A.E., McClaugherty, C.A., Rayburn, L., Repert, D., Weiland, T., 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology 74, 1586–1593.
Sinsabaugh, R.L., Linkins, A.E., 1988. Adsorption of cellulase components by leaf litter. Soil Biol. Biochem. 20 (6), 927–931.
Sinsabaugh, R.L., Reynolds, H., Long, T.M., 2000. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biol. Biochem. 32 (14), 2095–2097.
Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton, New Jersey, USA.
Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in Terrestrial Ecosystems. Blackwell Scientific Publications, Oxford, UK.

Tate, R.L., 1995. Soil Microbiology. Wiley, New York.
Torres, I.F., Bastida, F., Hernández, T., Bombach, P., Richnow, H.H., García, C., 2014. The role of lignin and cellulose in the carbon-cycling of degraded soils under semiarid climate and their relation to microbial biomass. Soil Biol. Biochem. 75, 152–160.
Vinolas, L.C., Vallejo, V.R., Jones, D.L., 2001. Control of amino acid mineralization and microbial metabolism by temperature. Soil Biol. Biochem. 33 (7-8), 1137–1140.
Waldrop, M.P., Firestone, M.K., 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138 (2), 275–284.
Wu, J., Brookes, P.C., Jenkinson, D.S., 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biol. Biochem. 25 (10), 1435–1441.
Wutzler, T., Zaehle, S., Schrumpf, M., Ahrens, B., Reichstein, M., 2017. Adaptation of microbial resource allocation affects modelled long term soil organic matter and nutrient cycling. Soil Biol. Biochem. 115, 322–366. https://doi.org/10.1016/j.soilbio.2017.08.031.
Xie, H., Li, J., Zhu, P., Peng, C., Wang, J., He, H., Zhang, X., 2014. Long-term manure amendments enhance neutral sugar accumulation in bulk soil and particulate organic matter in a mollisol. Soil Biol. Biochem. 78, 45–53.
Xu, X., Schimel, J.P., Thornton, P.E., Song, X., Yuan, F., Goswami, S., Bardgett, R., 2014. Substrate and environmental controls on microbial assimilation of soil organic carbon: a framework for Earth system models. Ecol. Lett. 17 (5), 547–555.
Zak, D.R., Kling, G.W., 2006. Microbial community composition and function across an arctic tundra landscape. Ecology 87 (7), 1659–1670.
Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecol. Monogr. 85 (2), 133–155.