Substrate quality alters microbial mineralization of added substrate and soil organic carbon

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Abstract. The rate and extent of decomposition of soil organic carbon (SOC) is dependent on substrate chemistry and microbial dynamics. Our objectives were to understand the influence of substrate chemistry on microbial processing of carbon (C), and to use model fitting to quantify differences in pool sizes and mineralization rates. We conducted an incubation experiment for 270 days using four uniformly-labeled ^{14}C substrates (glucose, starch, cinnamic acid and stearic acid) on four different soils (a temperate Mollisol, a tropical Ultisol, a sub-arctic Andisol, and an arctic Gelisol). The ^{14}C labeling enabled us to separate CO_2 respired from added substrates and from native SOC. Microbial gene copy numbers were quantified at days 4, 30 and 270 using quantitative polymerase chain reaction (qPCR). Substrate C respiration was always higher for glucose than other substrates. Soils with cinnamic and stearic acid lost more native SOC than glucose- and starch-amended soils, despite an initial delay in respiration. Cinnamic and stearic acid amendments also exhibited higher fungal gene copy numbers at the end of incubation. We found that 270 days was sufficient to model decomposition of simple substrates (glucose and starch) with three pools, but was insufficient for more complex substrates (cinnamic and stearic acid) and native SOC. This study reveals that substrate quality imparts considerable control on microbial decomposition of newly added and native SOC, and demonstrates the need for multi-year incubation experiments to constrain decomposition parameters for the most recalcitrant fractions of SOC and added substrates.

1 Introduction

The chemistry of carbon (C) inputs into soils influences the rate and extent of microbial decomposition of soil organic carbon (SOC) (Schmidt et al., 2011; Schnitzer and Monreal,

2011). Three hypotheses are used to explain the decomposition of fresh C according to chemistry (Wickings et al., 2012): (i) chemical convergence, (ii) initial litter quality, and (iii) decomposer control. The chemical convergence hypothesis suggests that regardless of the differences in substrate quality and microbial diversity, all C substrates undergo decomposition through a limited number of biochemical pathways and reactions resulting in SOC of homogeneous chemistry (McGill, 2007; Fierer et al., 2009) and it supports the general understanding that simple sugars and amino acids are preferentially decomposed over complex lignin and ligno-cellulose. However, recent studies have also identified simple biopolymers of plant and microbial origin in the stabilized SOC (Sutton and Sposito, 2005; Kelleher and Simpson, 2006). According to the initial litter quality hypothesis, the chemical composition of substrates at the start of the decomposition process (e.g. leaf litter) exhibits a strong influence on decomposition rate and the chemistry of stabilized SOC (Angers and Mehuys, 1990; Berg and McClaugherty, 2008). The decomposer control hypothesis suggests that distinct decomposer communities impose constraints on substrate decomposition regardless of the difference in quality of substrate and stage of decomposition. Wickings et al. (2012) analyzed these three hypotheses through a long-term litter decomposition experiment and found experimental evidence for an interactive influence of both 'initial litter quality hypothesis' and 'decomposer control hypothesis' on the chemistry of decomposing letter. While these two hypotheses appear to be complimentary in nature, there have few studies that expressly examine the combined influence of initial substrate quality and the decomposer community on the decomposition of C inputs leading to SOC formation and stabilization.

Most past studies addressed the initial C substrate quality effect by adding isotopically-labeled and/or chemically distinct plant litters to soils in laboratory microcosms. Labeling with

^{13}C or ^{14}C isotopes allows separate quantification of SOC-derived CO_2 and substrate-derived CO_2, and specifically resolves the effects of substrate additions on SOC turnover (Kuzyakov and Cheng, 2001; Leake et al., 2006; Williams et al., 2006; Werth and Kuzyakov, 2008). Isotopically-labeled natural plant litter, however, can't be used to identify the role of specific litter constituents on SOC dynamics (Grayston et al. 1998; Loreau, 2001). One way to overcome this issue is to apply isotopically-labeled C compounds representing different constituents of plant residues, e.g. simple sugars, polysaccharides, proteins, lipids, and/or aromatic compounds to observe their direct effect on SOC decomposition (e.g. Brant et al., 2006; Hoyle et al., 2008; Schneckenberger et al., 2008; Strahm and Harrison, 2008; de Graaff et al., 2010). These studies indicated increased, decreased or no change in SOC decomposition dynamics due to the addition of substrates compared to unamended control treatments. Also, most of these studies used only labile C compounds such as simple sugars and organic acids as C amendments, and did not account for other, relatively more recalcitrant C compounds such as lignin, fatty acids, lipids etc. Therefore, more studies with isotopically-labeled substrate additions are needed to determine the role of initial litter quality on SOC decomposition.

In accordance with the decomposer community hypothesis, the magnitude of SOC change largely depends on the abundance and diversity of soil microbial communities (Fontaine et al., 2005). Bacteria and fungi are the major drivers of substrate and SOC decomposition comprising more than 90% of the soil microbial biomass, and clear evidence exists that these groups function differently in the decomposition process (de Graaff et al., 2010). There is a general understanding that easily available simple C compounds are taken up by the fast growing r-strategists in the early stages of decomposition, while in the later stages slow-growing k-strategists break down more recalcitrant C, i.e., compounds having higher thermodynamic

activation energies (Wardle et al., 2002; Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008). Among the r-strategists, bacteria are mostly considered responsible for utilizing labile C sources immediately after their addition to soils (Paterson et al., 2007; Moore-Kucera and Dick, 2008). Fungi are commonly regarded as k-strategists utilizing C from more recalcitrant substrates (Otten et al., 2001). However, this general paradigm has been challenged by other studies. For example, Nottingham et al. (2009) reported that gram-negative bacteria also belong to k-strategists and are responsible for the decomposition of complex C compounds, and Rinnan and Bååth (2009) did not find evidence that bacteria were more efficient in utilizing simple compounds than fungi. Evaluation of the interplay of these life-history strategies on SOC turnover across a suite of substrates, soils and microbial communities is still lacking and is essential to resolve the role of the decomposer community on SOC dynamics.

Lab-scale incubation studies have been instrumental to quantify the influence of initial litter quality and decomposer community by modeling SOC pool sizes and mineralization rates. Although laboratory incubations deviate from natural ecosystem environments in terms of continuous C input, microbial community structure and environmental conditions, they help to isolate specific mechanisms by systematically eliminating variations in certain environmental variables. Since there is no continuous C input during the course of the experiment, incubation studies can be used to quantify the mineralization kinetics of different fractions of C pools according to different types of substrate addition (Schädel et al., 2013). Statistical models are used to estimate the sizes and rates of SOC pools by curve fitting. Within these constraints, total SOC is generally divided into three pools with fast, intermediate and slow mineralization rates (Trumbore, 1997; Krull et al., 2003). The terminology, definitions and measurement techniques of these pools, however, vary widely in the literature. The lack of experimental data using

multiple substrates in long-term incubation experiments, however, limits understanding of the role of substrate complexity and decomposer community (von Lützow and Kögel-Knabner, 2009; Schädel et al., 2013).

In this paper we used long-term incubations to investigate how the chemistry of added C substrates affected mineralization of the substrate C and of the SOC, and the composition of the decomposer community in several different soils. We hypothesized that: (i) cumulative respiration of substrate C and native C would be higher when soils are amended with easily metabolized substrates compared to relatively more complex substrates, and that (ii) both incubation time and the relative recalcitrance of the added substrate would favor soil fungi over bacteria. To test these hypotheses, we conducted a long-term (270 day) laboratory incubation experiment using four different uniformly-labeled ^{14}C substrates (monosaccharide, polysaccharide, aromatic, fatty acid). The ^{14}C labeling enabled us to separate substrate-derived CO_2 from native SOC-derived CO_2. We tested the effect of different substrate additions on substrate and native C respiration using a first order exponential decay model, and utilized quantitative polymerase chain reaction (qPCR) to compare bacterial and fungal gene copy numbers. Finally, we incubated four different soils that spanned a wide range in climate, soil development, and type and quantity of organic C inputs.

2 Materials and methods

2.1 Soil sampling and characterization

Soils were collected from four contrasting climatic zones- temperate, tropical, sub-arctic and arctic. The selected soils are from major soil orders of the respective climatic regions: the

Mollisol (temperate), the Ultisol (tropical), the Andisol (sub-arctic), and the Gelisol (arctic) (Table 1). Multiple soil cores were collected randomly from each location to a depth of 15 cm, pooled to form a composite sample per location and sieved to <2 mm. Subsamples (n=3) of the soils were taken for the determination of organic C, total N, microbial biomass C (MBC), soil pH, and soil texture (Table 1). Organic C and total N concentrations were determined by combustion method using a Leco combustion analyzer (Leco Corp., St. Joseph, MI) (Nelson and Sommers, 1996) after removing the inorganic C by treating with 3*M* HCl for 1 hr. Determination of MBC was conducted by the chloroform fumigation extraction method (Vance et al., 1987). Soil pH was determined by shaking 1 part soil in 2 parts Milli-Q (MQ) water and measuring the pH of the supernatant (Thomas, 1996), and soil texture was determined by the bouyoucos hydrometer method (Gee and Or, 2002).

2.2 Carbon substrates

Four uniformly-labeled ^{14}C substrates were used: glucose, starch, cinnamic acid and stearic acid, representing several dominant C compounds present in plant litter and SOC, and spanning a range of chemical lability. Glucose is a common simple sugar and starch is a common polysaccharide in plant residues, cinnamic acid contains an aromatic ring and is a common product of lignin depolymerization, and stearic acid represents a fatty acid (Orwin et al., 2006; Rinnan and Bååth, 2009). Similar to Orwin et al. (2006), we selected compounds containing only C, hydrogen, and oxygen and lacking nutrient elements such as nitrogen and phosphorus. These nutrients are expected to cause confounding effects on microbial activities and C decomposition (Orwin et al., 2006). Availability in uniformly-labeled ^{14}C form (U-^{14}C) was also another criterion for the compound selection.

2.3 Incubation experiments

We used five control (unamended) replicates of each of 4 soils for measuring native SOC respiration. Two replicates were destructively harvested at days 4 and 30 and stored at -20˚C for microbial community analysis. The three remaining replicates were monitored for respiration until they were destructively harvested for community analysis at 270 days. An identical scheme was used for the 4 different substrates to measure $^{14}CO_2$ evolved from decomposition of substrate and CO_2 evolved from native SOC. Our initial experiment thus had 4 soils each having 5 controls and five ^{14}C substrate additions, using 4 different substrates. Though we could include only one replicate for the destructive sampling at day 4 and day 30 due to limitations of space, soil, and ^{14}C substrate, we conducted three analytical replicates of the microbial community measurements for these sampling times, and three experimental replicates for the 270 day sampling time.

For the substrate addition experiments, 25 g (oven-dry basis) soils were amended with 0.4 mg C g^{-1} soil substrates which were labeled with 296 Becquerel g^{-1} soil U-^{14}C substrate. The substrates were added in dissolved form and mixed well with the soil using a spatula. The final moisture content of substrate amended and unamended samples were maintained at 50% WHC with MQ water. The solvents were MQ water for glucose and starch, ethanol for cinnamic acid and toluene for stearic acid. Organic solvents were used for cinnamic acid and stearic acid because these compounds are sparingly soluble in water. We introduced only a small amount of organic solvents to the samples (4 µL ethanol g^{-1} soil and 6 µL toluene g^{-1} soil) and our preliminary experiments revealed that the solvents did not influence the microbial activities (Fig. S1

2.4 Measurement of CO_2 respiration

Specimen cups containing the substrate amended and unamended control soils were placed in 1L, wide mouthed glass jars, along with a glass vial containing 17 ml of 0.5 N NaOH solution to trap the evolved CO_2. The jars were tightly closed and incubated in the dark at 20 ºC for up to 270 days in a temperature and humidity controlled room. The NaOH solution was exchanged 15 times during the experiment at daily to weekly intervals in the first two months and monthly intervals thereafter. The jars were sufficiently ventilated each time when they were opened for NaOH solution exchange in order to avoid anaerobic conditions inside the jar.

The amount of total C respiration is defined as the sum of SOC-derived CO_2 and substrate-derived $^{14}CO_2$, where the control (unamended) samples have no contribution from substrate. Total mineralized CO_2 was determined by titrating an aliquot of NaOH solution collected at each sampling time with 0.5 N HCl by an automatic titrator (Metrohm USA). Before the titration, the CO_2 collected in NaOH solution was precipitated as barium carbonate ($BaCO_3$) by adding 2 ml 10% barium chloride ($BaCl_2$). The volume of acid needed to neutralize the remaining NaOH (unreacted with CO_2) was determined by the titration, which was used to calculate the concentration of CO_2 trapped in the NaOH solution (Zibilske, 1994). Evolution of substrate C was determined by measuring the activity of $^{14}CO_2$ trapped in NaOH solution collected from the substrate amended samples with a Packard Tri-Carb Liquid Scintillation Counter (LSC) after mixing 5 ml of the NaOH solution with 10 ml of the scintillation cocktail Ultima Gold XR (PerkinElmer). The CO_2 derived from SOC for the substrate-amended samples was calculated by subtracting substrate-derived $^{14}CO_2$ from the total CO_2.

2.5 Microbial gene copy numbers

Microbial DNA extraction was conducted with 0.25 g of moist soil using the PowerSoil DNA Isolation Kit (MOBIO Laboratories, Inc., CA, USA). The abundance of the ribosomal RNA (rRNA) genes was determined by quantitative real time polymerase chain reaction (qPCR) on a CFX96TM Real-Time PCR Detection System (Bio-Rad Laboratories, CA, USA) with group specific ribosomal DNA gene primers using iQ SYBR Green Supermix (Bio-Rad, CA, USA). A small segment of the sample DNA was amplified using primer pairs that targeted the conserved region of the rRNA. Gene copy numbers for bacteria, fungi and archaea were determined in analytical triplicates using standard curves constructed from group specific microorganisms. The primers, PCR reaction conditions, composition of the reaction mixture and the pure cultures used for preparing the standard curves are described in Table S1.

2.6 Exponential decay modeling

The respiration data (both the substrate C and SOC) were tested using a double and a triple pool first order exponential decay model (Farrar et al., 2012):

$$\text{Double pool model: } C_t = C_1(e^{-k_1 t}) + C_2\,(e^{-k_2 t}) \quad (1)$$

$$\text{Triple pool model: } C_t = C_1(e^{-k_1 t}) + C_2\,(e^{-k_2 t}) + C_3\,(e^{-k_3 t}) \quad (2)$$

where C_t is the total substrate C (in terms of % of added substrate C) or total SOC (in terms of % of initial SOC) remaining in time t, C_1, C_2, and C_3 are pool sizes, and k_1, k_2 and k_3 are associated mineralization rates. For the double pool model, C_1 and C_2 are defined as fast and intermediate pools, respectively, and for triple pool model, C_1, C_2 and C_3 are defined as fast, intermediate and

slow pools, respectively. For each set of data, multiple pool models were fit using Sigma plot v11 (Systat Software Inc., IL, USA) and dependency values and r^2 for fit parameters were calculated. We followed two criteria to determine the best fits as outlined in Farrar et al. (2012): (i) dependencies less than 0.98, and (ii) a statistically greater r^2 over a lower-order fit.

2.7 Statistical analysis

Statistical analyses were conducted using SAS software (SAS Institute Inc., 2002). The effect of substrate type on substrate-derived and SOC-derived respiration was determined by repeated measures analysis using the PROC MIXED option of SAS with incubation length considered as the repeated measure with autoregressive 1 covariance structure. The repeated measures analysis with the PROC MIXED option of SAS is analogous to the generalized linear model analysis with the PROC GLM option of SAS, except that the former allows modeling of the covariance structure of the dataset to account for unevenly spaced sampling dates (Littel et al., 1996; Schaeffer et al., 2007). *Post hoc* comparisons for determining the effect of substrate types on respiration, and modeled mineralization parameters (pool sizes and rates) in each soil were performed using PROC GLM of SAS. The treatment effects were separated using the Fisher's protected least significant difference (LSD) test. T-test was performed to determine if fungal to bacterial (F:B) gene copy ratio upon substrate addition was significantly different from F:B ratio of unamended controls at each time point. In all statistical tests, the mean differences were considered significant at $P \leq 0.05$. Error bars are represented as one standard error of the mean.

3 Results

3.1 Substrate-derived C respiration

There was a significant effect of substrate chemistry on substrate mineralization ($P \leq 0.05$), with respiration from glucose addition being the greatest (Fig. 1). Respiration rate was highly variable among substrates in the first several days of incubation. After day 2 of incubation, the proportion of added C respired as CO_2 for different soils was 18 to 28% from glucose, 12 to 16% from starch, 0.2 to 5% from cinnamic acid and 0.1 to 0.4% from stearic acid. Thus, a considerable initial delay was observed in the mineralization of C from cinnamic acid and stearic acid as compared to glucose and starch. At the end of incubation, cumulative respiration for different soils was 52 to 60% of added C for glucose, 39 to 49% for starch, 33 to 53 % for cinnamic acid and 43 to 57 % for stearic acid. Respiration from substrates varied within a narrow range for the Mollisol and the Andisol throughout the course of incubation compared to the Ultisol and the Gelisol. At the end of incubation, the proportion of substrate C respired for all substrates combines was 41 to 50% for the Mollisol, 43 to 54% for the Andisol, 33 to 57% for the Ultisol and 39 to 60% for the Gelisol.

3.2 SOC-derived C respiration

The cumulative amount of native SOC mineralized at the end of experiments with unamended soils varied from 2.4 to 4.1 mg C g^{-1} across the soils and substrate types (Table 2). Adding substrates significantly affected the amount of native SOC mineralized from the Ultisol, the

Andisol and the Gelisol, but not from the Mollisol (Table 2, Fig. S2). Contrary to our hypothesis, cinnamic acid and stearic acid additions resulted in mineralization of more native SOC than from unamended control in all soils except the Mollisol. Compared to the unamended control, cinnamic acid treatment caused 24% more mineralization of native SOC in the Ultisol, 36% more in the Andisol, and 20% more in the Gelisol. Likewise, stearic acid addition caused 28% more SOC mineralization in the Ultisol and the Andisol, and 30% more in the Gelisol. Cumulative SOC mineralization from glucose and starch treated soils was statistically similar to unamended soils (Table 2).

3.3 Microbial community composition

The fungal:bacterial (F:B) ratios were calculated from the fungal and bacterial gene copy numbers measured by qPCR (Fig. S3, S4). F:B >> 1 indicates fungal dominance and F:B << 1 indicates bacterial dominance. To compare the F:B ratios from the substrate amended and unamended samples, we calculated the difference ($F:B_{amended}$ - $F:B_{unamended}$) at each sampling point (day 4, 30 and 270) (Fig. 2). Positive values indicate greater fungal (and lesser bacterial) numbers in amended versus unamended soils, and negative values indicate smaller fungal (and greater bacterial) numbers in amended versus unamended soils. Positive values were nearly always observed for the Ultisol, the Andisol, and the Gelisol, and these values became more positive over time, indicating increasing fungal dominance. At day 4, the difference between F:B ratios between substrate amended and unamended soils was small, except for glucose addition to the Mollisol and the Ultisol which showed fungal dominance. Cinnamic acid and stearic acid addition exhibited enhanced fungal dominance by day 270 except for the Mollisol. Archaeal

gene copy numbers were also measured by qPCR and they were the lowest among the microbial groups for all substrate types, soil orders and sampling times (data not shown). There was no evident influence of substrate addition or length of incubation on archaeal numbers.

3.4 Pools and rates associated with respiration

Native SOC respiration was best modeled by the double pool exponential decay model. Irrespective of the substrate treatments, the lowest proportion of the initial SOC was assigned to labile pool (Pool 1) for the Andisol compared to other soils (Fig. 3A). The size of Pool 1 was greater for stearic acid and cinnamic acid amended soils than for control soils and soils with other substrates. For the Gelisol and the Ultisol, cinnamic acid and stearic acid addition yielded lower mineralization rate k_1 associated with Pool 1, while no difference was observed for the Mollisol or the Andisol (Fig. 3B). The mineralization rate k_2 corresponding to intermediate pool (Pool 2) was statistically similar among the substrates for all soils, however, there was a notable decrease in k_2 for the Andisol in comparison with other soils (Fig. 3C).

Modeling of substrate-derived respiration data was strongly dependent on substrate chemistry: a triple pool exponential decay model was the best fit for the substrate-derived C respiration following glucose and starch amendments, whereas a double pool model was the best fit following cinnamic acid and stearic acid amendment (Fig. 4). When comparing modeled C pools from cinnamic/stearic acid to glucose/starch amendments, Pool 1 of cinnamic/strearic acid amended soils mostly equals or exceeds the combined size of Pool 1 and Pool 2 modeled from glucose and starch respiration (Fig. 4A). The mineralization rate k_1 associated with Pool 1 following glucose and starch amendments was one or two orders of magnitude greater than the

corresponding k_2, which again was considerably greater than k_3 (Fig. 4B, 4C, 4D). Mineralization rate k_1 of cinnamic acid and stearic acid respiration was closer to the k_2 of glucose and starch respiration, and the k_2 following cinnamic acid and stearic acid respiration was equal to or lower than k_3 following glucose and starch addition. Since two types of models were needed to best fit the respiration data of two sets of substrates ~~(3 pool model for glucose and starch, and 2 pool model for cinnamic acid and stearic acid)~~, a statistical comparison of mineralization kinetics across substrate types was not conducted. Pool sizes and rates showed variations across soil types, but no overall consistent patterns were observed.

4 Discussion

4.1 Substrate-derived C respiration

In accordance with our hypothesis, substrate C mineralization rate and extent were influenced by initial substrate quality (Fig. 1). Indeed, the greatest mineralization of substrate C occurred following glucose addition (52-60% of added C); and in the initial days after substrate addition, we observed more rapid mineralization of C from glucose and starch than from cinnamic acid and stearic acid (Fig. 1). Our results with glucose and starch was quantitatively similar to previous studies (Bremer and Kuikman, 1997; Jones and Murphy, 2007; Hoyle et al., 2008) and in a similar experiment, Orwin et al. (2006) found that CO_2 respiration from sugars was greater than respiration from fatty acids and tannin. Considerably higher CO_2 efflux in the first three days of incubation was found when a synthetic root exudate cocktail containing 60% sugars, 35% organic acids and 2% amino acids was added to soils (de Graaff et al., 2010). The slower

349 degradation following starch addition in comparison to glucose addition in our study could be
350 due to the requirement of extracellular enzymes (α-glucosidase) for starch hydrolysis to occur
351 (Kelley et al., 2011; German et al., 2012), while glucose can be directly assimilated by microbes.
352 Contradictory to the general notion that the fast growing sugar feeders are composed mostly
353 of bacterial species (Paterson et al., 2007; Moore-Kucera and Dick, 2008), enhanced F:B values
354 at day 4 following glucose addition in our study indicates that some fungi responded quickly to
355 substrate addition (Broeckling et al., 2008; Chiginevaa et al., 2009; de Graaff et al., 2010).
356 Panikov (1995) and Rinnan and Bååth (2009) also observed fungal-controlled mineralization of
357 glucose in the initial phase of similar microcosm studies. Addition of a synthetic root exudate
358 mixture containing 60% simple sugars resulted in a higher proportion of fungal growth relative
359 to bacterial growth at day 3 (de Graaff et al., 2010).
360 Sugars and other easily assimilable substrates added to soil are used by microbes not only for
361 the production of energy and release of CO_2, but also for the biosynthesis of products including
362 extracellular enzymes, extracellular polysaccharides, cell wall polymers, storage compounds and
363 stress response compounds (Nguyen and Guckert, 2001; Dijkstra et al., 2011; Schimel and
364 Schaeffer, 2012). The proportion of C initially allocated for biosynthetic processes may take
365 more time to mineralize to CO_2. Consequently, we observed continued evolution of $^{14}CO_2$ even
366 after several months of incubation from all the added substrates (including the most labile
367 glucose), albeit at a slower rate. Therefore it is very likely that part of the added sugars may have
368 been used as biosynthetic precursors and those microbial byproducts contributed to the evolution
369 of $^{14}CO_2$ during the later stages of incubation.
370 The metabolism of C substrates in soil is a function of microbial community structure, the
371 relative access that different groups of microbes have to these substrates (Schimel and Schaeffer,

2012). Relative controls imposed by soil biology and physics, however, are not readily apparent. We observed that mineralization of C from cinnamic acid and stearic acid was delayed for several days (Fig. 1). However, this delay was not due to the decreased abundance of microbial activity because native SOC mineralization was similar to control. Specialized microorganisms might be responsible for the mineralization of these relatively complex compounds, and these organisms were either low in abundance in the beginning of the experiment, or the organisms simply took more time to consume and cycle these compounds. Degradation requires the production of specific extra-cellular enzymes before they can be utilized (German et al., 2011). Sorption to the soil mineral phase could be another reason for the delayed respiration, because our previous experiments showed considerably higher affinity of stearic acid to soil minerals in comparison with other compounds (Jagadamma et al., 2014). The eventual decomposition of stearic acid in this study, however, suggests that sorption did not protect stearic acid over longer time frames.

4.2 SOC-derived C respiration

We found that the chemical composition of substrates added to soils altered the stability of native SOC, but the results were different than what we originally hypothesized. Surprisingly, cumulative native SOC mineralization showed an increase due to cinnamic acid and stearic acid addition relative to glucose and starch additions and unamended soils (Table 2), and further, the increase in decomposition was only evident after several weeks of incubation (Fig. S2). Literature on substrate-controlled difference in SOC mineralization is scanty and the limited studies available mostly used simple sugars and organic acids as substrates to understand the

SOC mineralization process. In our study, we consider cinnamic acid and stearic acid as more complex C compounds than glucose and starch because of the higher hydrophobicity of both compounds, aromatic structure of cinnamic acid, and strong mineral sorption capacity of stearic acid (Orwin et al., 2006; Jagadamma et al., 2014). In a similar study, Brant et al. (2006) measured SOC mineralization following the addition of glucose, glutamate, oxalate and phenol from a forest soil in Oregon and found that more SOC was mineralized with oxalate and phenol addition compared to glucose and glutamate addition. We also found that cinnamic acid and stearic acid additions were associated with higher F:B gene copy ratios during the final stages of incubation relative to other substrates (Fig. 2). It is possible that the addition of cinnamic acid and stearic acid might have activated some specialized, but slow-growing fungal populations capable of decomposing more recalcitrant components of SOC at the later stages of incubation. Overall, our study reveals that both initial substrate quality and decomposer community are tightly linked and interactively influence the decomposition of both substrate and soil C.

4.3 Pools of carbon and rates of decomposition

Modeling of C pool sizes and mineralization rates from incubation-derived data are used for improved parameterization of ecosystem models. The cumulative CO_2 respiration following substrate addition was best described using a double or triple pool first order exponential decay model, for both amended and unamended soils (Chen et al., 2009; Farrar et al., 2012). The substrate C respiration following glucose and starch addition was best fit by a triple pool model (fast, intermediate and slow pools) and cinnamic acid and stearic acid additions were best fit by a double pool model, i.e., fast and intermediate pools (Fig. 4). Farrar et al. (2012) also reported

418 that a triple pool model was the best fit for the glucose derived CO_2. The need for two types of
419 models for sugars vs. complex compounds indicates that initial substrate quality hypothesis hold
420 true for the decomposition of C input (Wickings et al., 2012). The native SOC-derived CO_2 data
421 was best modeled using a double pool model regardless of the type of substrate addition (Fig. 3)
422 and the length of incubation experiment could be a determinant for the lack of effect of substrate
423 type on native C pool partitioning because incubation length reflects the contribution of more
424 recalcitrant pools in the total CO_2 efflux (Schädel et al., 2013). Shorter-term incubation data is
425 often dominated by the CO_2 from more labile C fractions. Using 385 days of decomposition data,
426 Schädel et al. (2013) did not find any improvement in the fit for SOC decomposition data when a
427 three pool model was used over a two pool model, and the dominance of the third pool became
428 more evident only after 230 days of study. Scharnagl et al. (2010) reported that decomposition
429 data from a 900-day incubation experiment was sufficient in constraining all the five C pools in
430 RothC model. In our study, within 270 days only 5 to 20% of initial SOC was lost across all soils
431 and substrate addition treatments (Table 2) and it appears that 270 day incubation was not long
432 enough to constrain parameters for the third native SOC pool. This differs from our substrate C
433 modeling in which three pools were used for glucose and starch but only two pools for cinnamic
434 acid and stearic acid. These findings support the need for more long-term studies using more
435 complex substrates. Substrate-specific mineralization kinetics are useful for refining the
436 decomposition rates and pools in C cycle models.
437
438 **5 Conclusions**
439
440 This study reveals that substrate quality imparts considerable control on microbial decomposition
441 of substrates and native OC, and also calls for multiple year incubation experiments to capture

442 the dynamics of the recalcitrant fraction of the OC pool. We found that even though complex
443 substrates (cinnamic acid and stearic acid) showed an initial delay in respiration compared to
444 simpler substrates (glucose and starch), complex substrates caused enhanced mineralization of
445 SOC at later stages of incubation with a concomitant increase in fungal abundance. However, the
446 length of incubation was not long enough to fully characterize decomposition kinetics of more
447 complex substrates (cinnamic acid and stearic acid) and native SOC. This study suggests the
448 need for more detailed experiments investigating the role of substrate quality on C
449 mineralization, and the need to design experiments to capture the dynamics of both the labile and
450 recalcitrant fraction in soils. Characterizing these dynamics is critical as anthropogenically-
451 induced changes in atmospheric CO_2 concentration and N deposition are predicted to alter the
452 quality of both above ground and below ground C input to soils. Thus, understanding the control
453 of substrate chemistry or quality on soil microbial composition and function will be useful to
454 predict the future impact of climate change on SOC dynamics.
455
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464

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687 **Table 1.** Sampling locations and pre-incubation soil properties.

Descriptions	Soils			
	Mollisol	Ultisol	Andisol	Gelisol
Sampling location	Batavia, Illinois, USA	Lavras, Minas Gerais, Brazil	Krýsuvíkurheiði, Reykjanes, Iceland	Fairbanks, Alaska, USA
Organic C (g kg^{-1})	29.8 ± 0.50	23.2 ± 1.2	74.5 ± 0.10	20.5 ± 0.10
Total N (g kg^{-1})	3.00 ± 0.02	1.97 ± 0.08	7.09 ± 1.08	1.32 ± 0.02
Microbial biomass C (mg kg^{-1})	640 ± 35	515 ± 42	856 ± 39	48 ± 2.30
pH (1soil:2H_2O)	7.64 ± 0.10	5.42 ± 0.01	5.84 ± 0.01	7.03 ± 0.10
Silt (g kg^{-1})	570 ± 30	170 ± 20	570 ± 46	790 ± 49
Clay (g kg^{-1})	350 ± 15	450 ± 32	120 ± 08	130 ± 11

688 Values are mean ± standard error (n=3).

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Table 2. Cumulative soil organic C (SOC) respiration after 270 days.

		SOC respiration	
Soils	Substrates	mg C g^{-1} soil	% of initial C lost
Mollisol	Unamended	4.1±0.21	13.9
	Glucose	4.0±0.07	13.3
	Starch	3.9±0.15	13.1
	Cinnamic acid	4.0±0.18	13.6
	Stearic acid	4.3±0.12	14.4
Ultisol	Unamended	3.5±0.13^{b}	15.3
	Glucose	3.6±0.15^{b}	15.6
	Starch	4.0±0.10^{ab}	17.4
	Cinnamic acid	4.4±0.23^{a}	19.0
	Stearic acid	4.5±0.04^{a}	19.6
Andisol	Unamended	3.4±0.15^{b}	5.3
	Glucose	3.5±0.04^{b}	5.5
	Starch	3.6±0.11^{b}	5.5
	Cinnamic acid	4.6±0.21^{a}	7.2
	Stearic acid	4.4±0.04^{a}	6.8
Gelisol	Unamended	2.4±0.6^{b}	11.8
	Glucose	2.7±0.17^{b}	12.9
	Starch	2.6±0.0^{b}	12.6
	Cinnamic acid	2.9±0.11^{ab}	14.2
	Stearic acid	3.1±0.11^{a}	15.3

Each value represents mean ± standard error (n=3). Different letters indicate significant mean differences among substrate addition treatments in each soil.

Figure captions

Fig. 1. Substrate C respiration in response to the addition of four substrates in Mollisol (A), Ultisol (B), Andisol (C), and Gelisol (D). Symbols represent proportion of added substrate C respired at each sampling time along with standard error bar (n=3).

Fig. 2. The difference in Fungal:Bacterial gene copy ratios between amended and unamended treatments ($F:B_{amended}$-$F:B_{unamened}$) in response to the addition of four substrates in Mollisol (A), Ultisol (B), Andisol (C), and Gelisol (D). * indicates that $F:B_{amended}$-$F:B_{unamended}$ is significantly different from zero.

Fig. 3. Effect of substrate types on native soil organic carbon mineralization parameters: pool sizes (A), mineralization rate k_1 associated with Pool 1 (B), and mineralization rate k_2 associated with Pool 2 (C).

Fig. 4. Effect of substrate types on substrate C mineralization parameters: pool sizes (A), mineralization rate k_1 (B), mineralization rate k_2 (C), mineralization rate k_3 (D). Pool sizes of glucose-C and starch-C respiration (fast, intermediate and slow pools) and their associated mineralization rates (k_1, k_2 and k_3) were best modeled by a triple pool model, and pool sizes of cinnamic acid-C and stearic acid-C respiration (fast and intermediate pools) and their associated mineralization rates (k_1 and k_2) were best modeled by a double pool model. Bars are mean ± standard error (n=3).

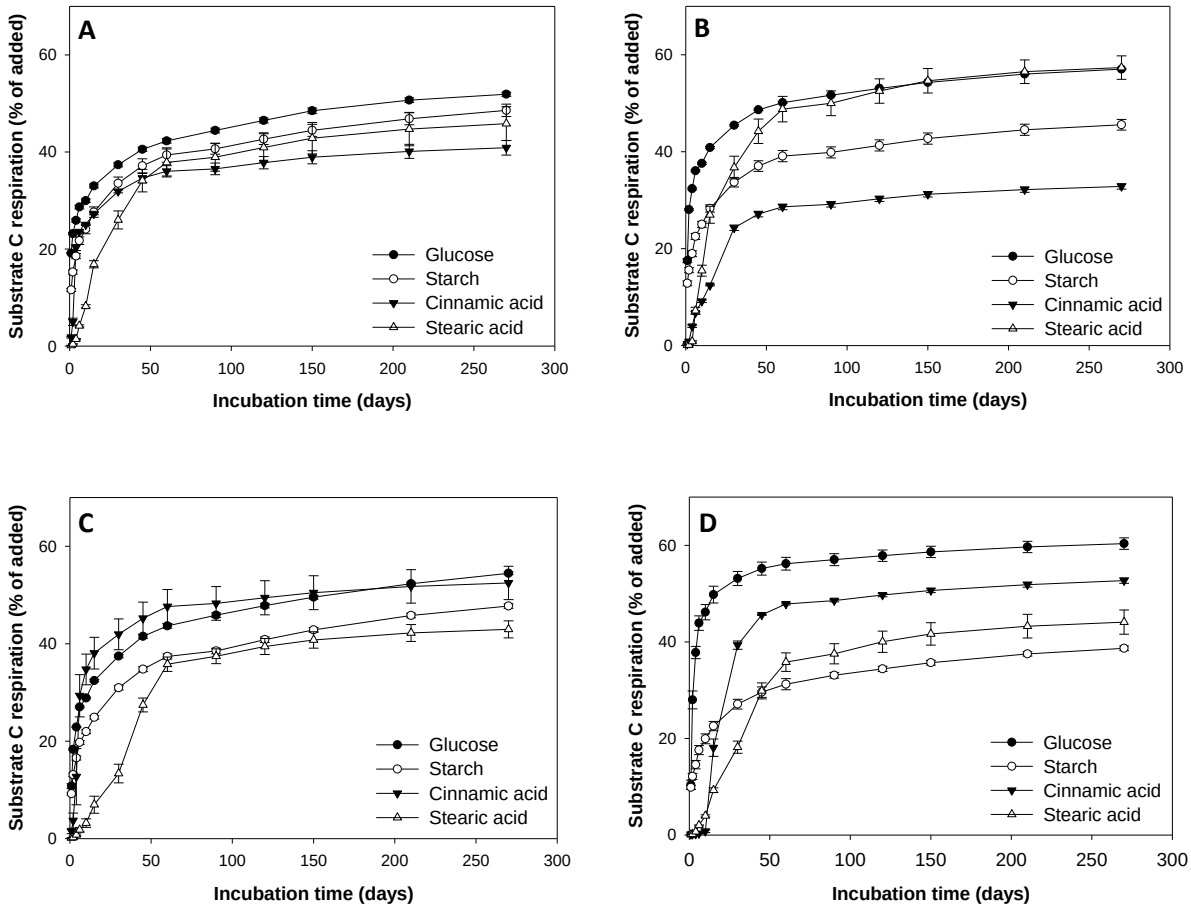

A
B
C
D
Substrate C respiration (% of added)
Incubation time (days)
Glucose
Starch
Cinnamic acid
Stearic acid
0
20
40
60
0
50
100
150
200
250
300

Fig. 1.

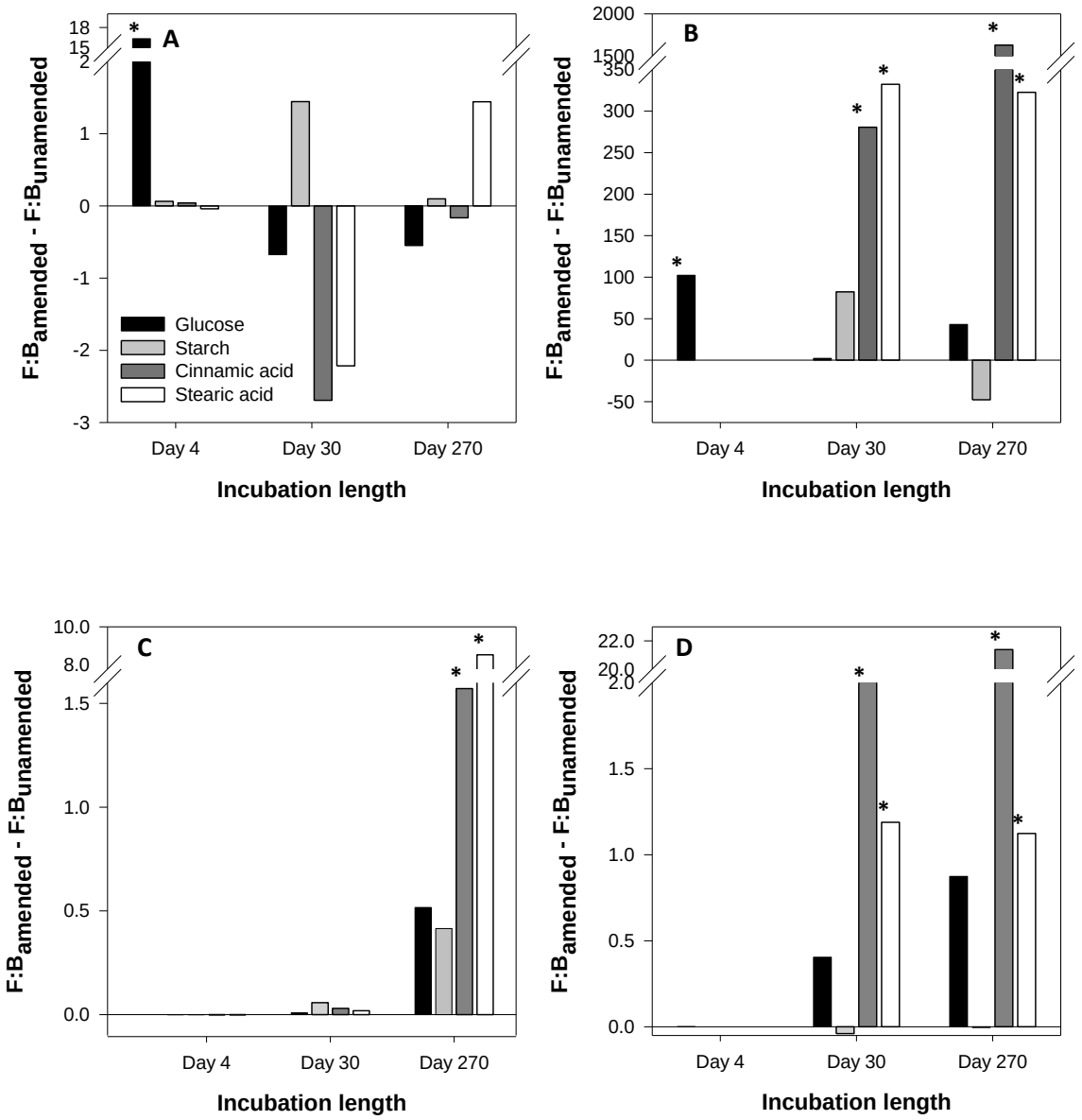
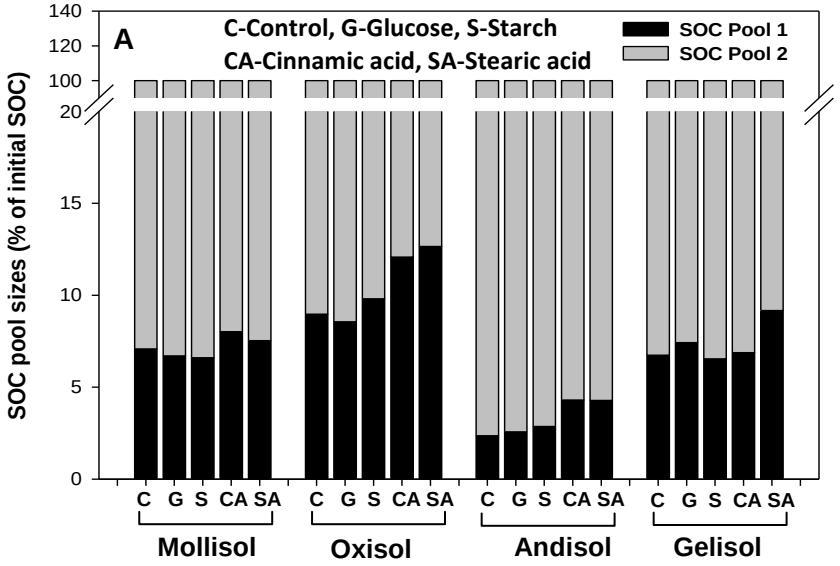

A
B
C
D
Glucose
Starch
Cinnamic acid
Stearic acid
F:Bamended - F:Bunamended
Day 4
Day 30
Day 270
Incubation length

Fig. 2.



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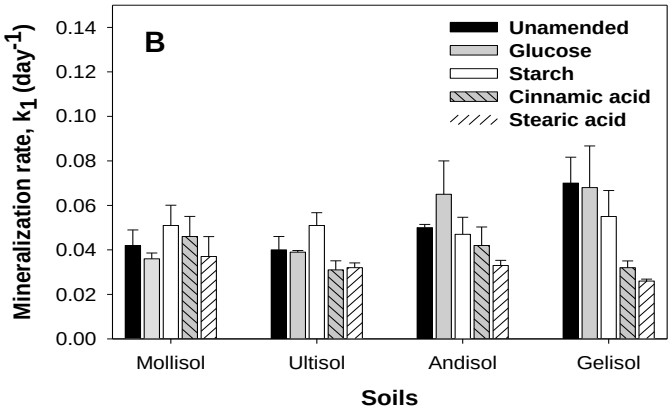

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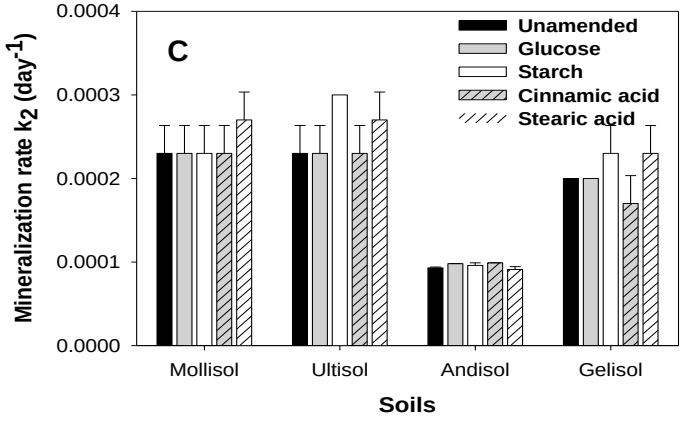

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754 **Fig. 3**

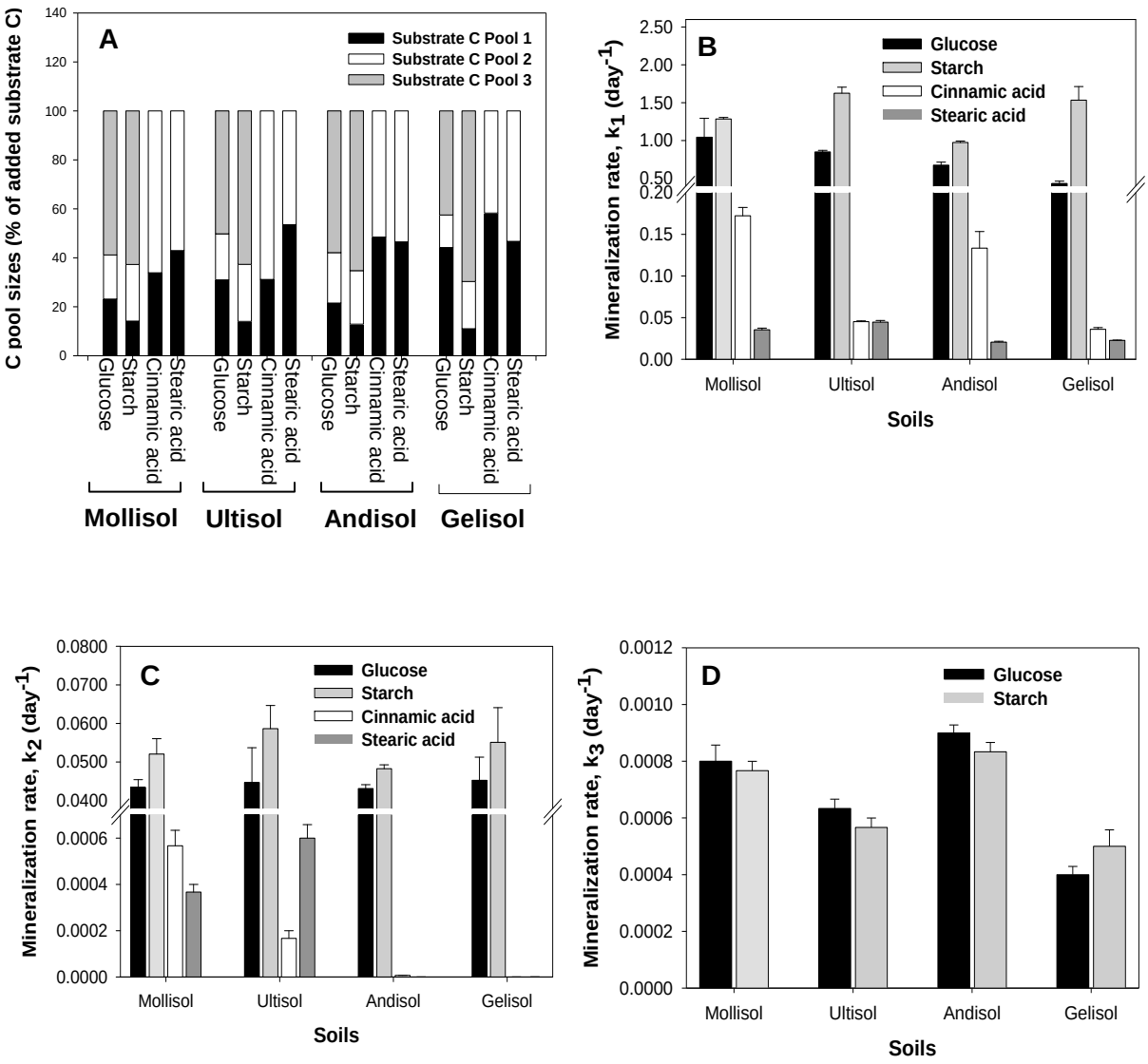
A
Substrate C Pool 1
Substrate C Pool 2
Substrate C Pool 3
C pool sizes (% of added substrate C)
Glucose
Starch
Cinnamic acid
Stearic acid
Mollisol
Ultisol
Andisol
Gelisol
B
Glucose
Starch
Cinnamic acid
Stearic acid
Mineralization rate, k1 (day-1)
Soils
C
Mineralization rate, k2 (day-1)
D
Mineralization rate, k3 (day-1)

Fig. 4.