1 **Substrate quality influences organic matter accumulation in the soil silt and clay**
2 **fraction**

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Abstract

Substrate quality impacts the rate of microbial decomposition of soil organic matter (OM), with higher quality substrates leading to faster rates of decomposition. Since OM decomposition is the opposite of OM stabilization, one might presume higher quality substrates would lead to less OM stabilization. Yet, there is growing evidence that C stabilized in the soil silt and clay fractions is preferentially derived from microbial metabolites. We hypothesized that the decomposition of higher quality substrates would increase silt and clay-sized (S)OM pools despite higher initial mineralization rates. Soils low in initial organic carbon were incubated for 139 d with substrates spanning a range of quality/lability including: (a) bermudagrass forage cut after 14d, 21d, 28d, 35d, and 42d of re-growth, and (b) ruminal digesta produced from these forages. We then monitored the production of CO₂ as well as the carbon abundance and isotopic composition in the bulk, silt, and clay fractions. Undigested forage was respired at higher initial rates than ruminal digesta and resulted in more carbon (C) and nitrogen (N) in the clay fraction. Overall, substrate quality—assessed as the ratio of neutral detergent fiber (NDF-cellulose, hemicellulose, and lignin) to crude protein (CP)—was directly related to decomposition kinetics with higher substrate quality resulting in more silt and clay C. These findings provide evidence that substrate quality, as a driver of microbial response, can control the flow of C and N to silt and clay fractions where there is the potential for interactions with mineral surfaces to greatly increase C and N residence times. Incorporating this concept into numerical models of SOM generation and turnover will likely improve projections of carbon dynamics in global change models.

42 **Keywords**

43 Soil organic matter
44 Soil carbon
45 Substrate quality
46 Microbial growth
47 Mineral stabilization
48 Mineral-organic associations

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50 **1. Introduction**

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52 Soil organic matter (SOM) represents the Earth's largest terrestrial carbon
53 reservoir (Jobbágy and Jackson, 2000). Plant and animal debris are the main biological
54 inputs to soil, comprising a molecularly complex mixture rich in polysaccharides and
55 lignin (Kögel-Knabner, 2002), which are physically and chemically broken down by soil
56 macro and micro-biota to form SOM. The breakdown of these inputs drives biological
57 processes in soils (Janzen, 2006) that provide many ecosystem services. The abundance
58 of SOM is a dynamic balance between the quantity of organic inputs, the rates and
59 efficiency of biomass synthesis during decomposition, and the interaction of transformed
60 organic molecules with soil mineral phases (Schlesinger and Bernhardt, 2013). These
61 same processes influence the magnitude of soil CO₂ flux to the atmosphere, as well as the
62 form of resulting SOM (Grandy and Neff, 2008).

63 Traditionally, scientists estimate litter decomposition rates based on the chemical
64 composition of the organic substrate and climatic variables (Meentemeyer, 1978; Zhang

et al., 2008; Prescott, 2010). The concept of substrate quality—indexed as lignin/N or C/N—has historically been implemented in soil carbon and nitrogen models as a means to partition new organic materials into pools of either rapidly or slowly decomposing organic matter, often with fixed first-order kinetic rate constants (i.e., k values) (Manzoni and Porporato, 2009). These approaches conceptually rely on the assumption that stable SOM is formed from more recalcitrant substrates (Prescott, 2010). However, recent observations have shifted the paradigm for explaining SOM persistence from one based on molecular recalcitrance alone (Kleber and Johnson, 2010; Dungait et al., 2012) to one driven by ecosystem properties, especially physiochemical interactions such as the stabilization of SOM via sorption to mineral surfaces (Schmidt et al., 2011; Kleber et al., 2015; Lehmann and Kleber, 2015).

While there is a large body of literature relating substrate chemistry to the initial decomposition rates, currently there is little data linking the quality of added substrates to changes in the more stable silt and clay fractions of soil (von Lützow et al., 2006; Rubino et al., 2007; Kleber et al., 2011; Wieder et al., 2013). Though absent from many models, overall substrate use efficiency (SUE) of the active microbial community—as influenced by physiological (Beardmore et al., 2011) and external constraints (Manzoni et al., 2012)—governs the supply of microbial metabolites to the fine mineral fraction. According to the proposed Microbial Efficiency-Matrix Stabilization Framework (MEMS) (Cotrufo et al., 2013), microbially-labile substrates (Lekkerkerk et al., 1990; Hobbie, 2005) should stimulate microbial growth and provide more C and N in the form of microbial exudates and necromass to the soil mineral fraction. Several field studies have shown that inorganic nutrient amendments (N, P, S) increase microbial carbon SUE

as well as SOM formation and stabilization (Thiet et al., 2006; Kirkby et al., 2013). Litter C can be rapidly incorporated (< 8 h) into silt and clay fractions (Angers et al., 1997; Vogel et al., 2014; Hatton et al., 2015) with many studies suggesting that this incorporation is linked to microbial growth (Miltner et al., 2012; Bradford et al., 2013) as well as substrate quality and community structure (Williams et al., 2006).

Missing from this revised paradigm is how the capacity of the soil mineral matrix influences the process of SOM formation (Castellano et al., 2015). The fine mineral fraction (<20 μm) is purported to have the capacity to protect a discrete mass of SOM based on the mineral surface area (Hassink, 1997) and composition (Six et al., 2002). SOM will thus accumulate within the fine mineral fraction only until this capacity is reached (i.e., the mineral surface becomes saturated). The importance of litter quality to C stabilization may thus depend on how close the soil is to its theoretical C saturation limit. Castellano et al. (2015) hypothesized that litter quality will only impact mineral-associated SOM stocks when there is a substantial saturation deficit (Stewart et al., 2007). This may be one reason evidence has been provided both for (Kirchmann et al., 2004; Bradford et al., 2013; Putasso et al., 2013) and against (Voroney et al., 1989; Helfrich et al., 2008) substrate quality as a driver of the formation of mineral-associated SOM. Regardless, mineral stabilization of fresh litter inputs is already being introduced into the next generations of soil C models (Wieder et al., 2015).

We suggest the role of substrate quality on soil C stabilization could be best tested using low carbon soils that are far below their hypothetical C saturation (Six et al., 2002). We hypothesized that higher quality organic matter inputs would lead to more mineral associated C and N and test if *de novo* SOM was more or less biologically stable than the

initial SOM. To test this we incubated plant-derived substrates of varying initial quality in soil with a high potential for C accumulation (Machmuller et al., 2015) under optimized environmental conditions. We then monitored C mineralization as well as C and N accumulation as a function of soil particle size.

2. Methods

2.1 Substrate preparation

The substrate addition treatments were designed to mimic residue inputs likely to occur in a management intensive grazing (MiG) dairy system, which typically receives forage and manure inputs with a range of quality (see Machmuller et al., 2015 for management details). A single grass species, bermudagrass, was chosen as the substrate source. Ruminant digestion was chosen as a treatment, as manure dominates aboveground carbon inputs in the MiG system (Pal et al., 2012) and ruminant digestion represents a significant microbial processing event that impacts substrate quality. The ratio of fibrous materials to protein present in the animal's diet will impact the chemical composition in deposited manure (Chadwick et al., 2000; van Vliet et al., 2007). For the purposes of this study, substrate quality is defined as the ratio of neutral detergent fiber (NDF) content (a measure of the structural components: cellulose, hemicellulose, and lignin) to crude protein (CP). The ratio of NDF/CP represents the relative proportion of structural plant cell components vs. more nitrogen rich components. Lower NDF/CP values indicate higher substrate quality. Forage maturity is a secondary treatment variable, as plant

biochemical composition changes with age. Older forage tends to have lower protein content and higher lignin content (Waksman and Tenney, 1927; Hill et al., 1995). Thus, increasing maturity and digestion decrease substrate quality. A factorial combination of these treatments was used to design an incubation of substrates ranging in NDF/CP (Table 1, Fig 1).

A field of bermudagrass ('Tifton 85', *Cynodon dactylon* (L.) Pers. X C. *nlemfuensis* Vanderyst) was grown under field conditions at the University of Georgia Plant Sciences Farm in Watkinsville, GA from July to October of 2011. Harvest was initiated on September 1st, 2012 by mowing to a height of 5 cm and fertilizing with 100 kg/ha N. The forage was cut after 14, 21, 28, 35, and 42 d of re-growth to a height of ~5 cm above the soil, representing a range of grazing periods within the MiG dairy system. All herbage samples were immediately frozen, lyophilized, ground through a 1 mm screen using a Wiley Mill.

An anaerobic digester under continuous culture conditions with mixed ruminal bacteria was used to generate ruminal digesta from herbage (Jenkins et al., 2014). Each forage treatment was divided into three, 30 g subsamples and digested for a 7 d, including 4 d of acclimation and 3 d of overflow sampling. Dried forage samples were analyzed for nutrient content by Cumberland Valley Analytical Laboratories (Maugansville, MD). Analyses included crude protein (CP) (Horwitz, 2000) and NDF via the α -amylase method (Van Soest et al., 1991). Carbon and nitrogen content and isotopic composition was measured at the UGA Stable Isotope Ecology Laboratory (Athens, GA). Results indicate the ruminal digesta has C/N ratios and NDF contents similar to what could be

expected in manure produced from the respective feeds (van Vliet et al., 2007, Jost et al., 2013).

2.2 Site description and soil collection

Soil was collected from a MiG dairy located in Burke County, Georgia. The soil was a Fuquay loamy sand (Arenic Plinthic Kandiudult) and was located on a 1-5% slope within the coastal plain region of the state. Burke County has an average annual temperature of 19°C and annual precipitation of 1224 mm yr⁻¹. The site had been converted from row-crop agriculture to MiG dairy management 4 years prior to sampling. A more detailed description of the land use regimes can be found in Machmuller et al. (2015).

Soil was collected in September 2013 from a 0-10 cm A horizon. Samples were immediately placed in Ziploc bags, and kept on ice during transport. All soil was passed through a 5 mm sieve at field moisture and any large, visible roots, or plant materials were removed. The homogenized soil was stored at 4°C in the dark for 1 d prior to beginning the incubation. Subsamples from the homogenized soil (~20 g) were taken for measurement of water content by drying six replicates overnight at 105°C and pH was measured using a 1:2 soil:water ratio (Kalra, 1995).

Analysis of all samples (initial soils plus all treatments at the end of the incubation) showed the soil contained $84 \pm 1.5\%$ sand, $11 \pm 1.5\%$ silt and $2.7 \pm 0.2\%$ clay based on particle size analysis ($n = 55$, see Section 2.4 for method details). The initial soil C content was low ($0.66 \pm 0.01\%$ C, $n = 5$), of which 34% and 28% of that C was

contained in the silt and clay fractions, respectively (Table 2). The ratio of C/N generally declined with decreasing soil particle size. The mineral composition of bulk soil was dominated by quartz, with kaolinite and hydroxy-interlayered vermiculite in the clay fraction (SI Appendix I). Spectral fitting of Mössbauer data indicates that the majority of the iron in the clay fraction is in the form of Fe^{III} within Fe^{III}-(oxyhydr)oxides, with only 17% of the Fe^{III} present in aluminosilicate structures (SI Table. S.2). The resuspension and sedimentation procedure (see Section 2.4) removed 20.6% of the original clay fraction C. Thus, clay_r-C was 21.5% of total soil C at the start of the incubation (Table 2).

2.3 Incubation

A fully factorial experimental design was utilized to assess the impact of digestion (undigested herbage vs. ruminal digesta) as well as maturity (14d, 21d, 28d, 35d, 42d) on the decomposition of bermudagrass residues. Measurements included: i.) CO₂ production rates and calculations of the C-remaining through the course of decomposition ii.) the final bulk soil C and N stocks, and iii.) final soil C and N contained within silt (53 µm > x > 2 µm) and clay particle (< 2 µm) size fractions.

All treatments were exposed to a 139 d soil incubation at optimal decomposition parameters of 60% water filled pore space (WFPS) and a temperature of 30°C. Five replicates of each factorial combination (digestion by maturity) were included, except for two of the ruminal digesta treatments (14-d and 28-d) where insufficient material allowed for only three replicates (SI Table S.1). Treatments were arranged and analyzed in a randomized block design. Individual experimental units were initiated and sampled in a

202 staggered manner to allow sampling at the same time interval. Jars containing the soil
203 matrix but no substrate addition—here referred to as no addition controls—were used
204 along with empty jars to estimate CO₂ produced from the decomposition of native SOM
205 as well as ambient CO₂.

206 Fifty grams of field-moist homogenized soil was placed in a 60-mL round bottom
207 graduated glass bottle (Qorpak) to which 0.25 g of substrate was added and thoroughly
208 mixed. The mixture was then lightly packed down to a bulk density of ~1.4 g cm⁻³. Water
209 (18.2 Mohm-cm and in all references hereafter) was added to the mixture to reach 60%
210 WFPS. The graduated glass bottle was then placed inside of a pint glass jar with two
211 other 20-mL glass scintillation vials: one vial contained 10 mL of water to maintain soil
212 humidity; the other vial contained 10 mL of 1 M NaOH to trap evolved CO₂. The
213 incubation was initiated without a preincubation period, with the realization that this may
214 slightly overestimate active C pool decomposition rates (Paul et al., 2001).

215 Microcosms were removed from the incubator and sampled as described below.
216 Sampling involved the removal of 2 mL of the 1 M NaOH base trap and storage in a 2
217 mL plastic centrifuge tube containing an airtight O-ring seal. All jars were allowed to
218 equilibrate with ambient atmospheric conditions for 10 min to prevent anoxic conditions
219 upon resealing. At the end of this period, water was added to the soil/residue mixture to
220 maintain 60% WFPS, a fresh vial of 10 mL of 1 M NaOH was added, and the seal
221 replaced. Care was taken to adjust sampling times to prevent anoxic conditions and to
222 ensure the base trap never exceeded 60% of the CO₂ trapping potential (Zibilske, 1994).
223 All NaOH samples were stored with minimal headspace and analyzed on a Shimadzu
224 5050 TOC analyzer using the inorganic carbon (IC) analysis procedure (acidification

using 25% H₃PO₄). Dilutions maintained pH values above 12.75 to prevent loss of CO₂. Appropriate Na₂CO₃/NaHCO₃ standards and blanks were used for calibration and a check standard was run every 10 samples to assess machine drift.

2.4 Particle size fractionation

The initial soil (n = 5) and all incubated soil samples were size-fractionated to separate silt and clay. Prior to separation, 20 mL of 0.5% hexametaphosphate (HMP) was added to 25 g of moist soil to disperse soil particles (Kettler et al., 2001). Samples were shaken overnight and then centrifuged at 144.8 g for 55 s to separate the clay fraction [$<2\text{ }\mu\text{m}$ equivalent spherical diameter (e.s.d) as calculated using Stoke's Law] (Moore and Reynolds, 1997). The supernatant was removed, frozen, and lyophilized. Care was taken not to remove any floating particulate organic matter that was clearly larger than clay sized. The remaining solids were then resuspended with water and passed through a $53\text{ }\mu\text{m}$ sieve to separate the sand and silt fractions. All particle size fractions, as well as $\sim 2\text{ g}$ dry mass equivalent bulk soil, were frozen, lyophilized, and ball-milled to $< 250\text{ }\mu\text{m}$. All soil samples were analyzed for organic carbon and nitrogen concentration and isotopic composition on a CHN analyzer with a continuous-flow isotope-ratio mass spectrometer.

A subsample of each freeze-dried clay fraction ($\sim 42\text{ mg}$) was resuspended with 2 mL of water, recreating the original solid to solution ratio. The resuspended sample was then shaken for 5 min and then centrifuged at $20,8107\text{ g}$ for 15 min. All supernatant was then carefully decanted. This procedure was employed to remove any readily soluble carbon, such as dissolved organic carbon (DOC), that was extracted along with the

original solution containing particulates < 2 µm. Supernatants were analyzed for total C using a Shimadzu TOC analyzer, and used to produce a refined estimate of clay fraction C following the water rinse (hereafter referred to as the clay_r).

2.5 Calculations and statistical analyses

The mean mass of CO₂ collected in empty jar controls (n =5) was subtracted from all treatments at each time interval to account for background atmospheric CO₂ in the jars. We then calculated the remaining C in each jar (%C_{remaining}) based on the total soil C plus the added substrate.

The percentage of C-remaining was calculated as:

$$\%C_{\text{remaining}} = (\text{net } C_{\text{evolved}} / C_{\text{Initial}}) * 100 \quad (1)$$

Net C_{evolved} (mg) is calculated at each time (n) cumulatively after subtraction of ambient C (mg) trapped in empty control jars.

$$\text{Net } C_{\text{evolved}} = \sum_{t=0}^n (C_{\text{evolved}} - C_{\text{ambient}}) \quad (2)$$

Calculations of %C_{remaining} were based on total soil C—not the added substrate alone, as others have done (Johnson et al., 2007; Sanaullah et al., 2010; Walela et al., 2014)—because there was evidence of altered mineralization kinetics of pre-existing SOM at later points of the incubation (e.g., higher respiration rates from no-addition control treatments than addition treatments and large negative %C remaining values for some substrates).

Mineralization rates, C_{min} (mg C kg⁻¹ soil d⁻¹), were calculated as a measure of net C_{evolved} (mg) as a function of total soil mass (kg) including the mass of substrate addition,

$$C_{\text{min}} = \text{net } C_{\text{evolved}} / t \quad (3)$$

where t is the time interval (d).

Two separate MANOVA repeated measures analysis were used to assess treatment impacts on both %C_{remaining} and C_{min}. A fully factorial model was used with digestion (undigested herbage vs. ruminal digesta), maturity (14, 21, 38, 35, 42 d), and a digestion by maturity interaction term listed as fixed effects. A multivariate analysis was considered more appropriate in this case since a large departure from the assumption of sphericity was observed using Mauchley's test for sphericity. The Wilk's lambda test statistic was used to evaluate all within-subject effects. Means comparisons were conducted at each sampling interval on data sorted by significant treatment factors using either a Tukey HSD or student's t-test depending on the number of levels of the significant treatment.

A double exponential model was fitted on the %C_{remaining} data using equation (4) (Johnson et al., 2007):

$$C_t = C_a \exp(-k_a t) + C_s \exp(-k_s t) \quad (4)$$

where C_t is %C_{remaining} at time t , C_a is the initial percentage of C in the active pool, C_s is the initial percentage C in the slow C pool ($100 - C_a$), and k_a and k_s are the rate constants (%C d⁻¹) for the active and slow pools, respectively. The models were fit constraining all parameters to be positive. A two-way ANOVA model with an interaction term was used to analyze treatment effects on fitted C_a , C_s , k_a and k_s parameters.

Final response variables, such as C and N across particle size fractions as well as isotopic signatures (see SI Table S.7), were analyzed using a two-way ANOVA model with an interaction term. Pairwise comparisons were conducted based on significant model effects using a Tukey HSD or student's t-test. Final response variable data,

collated by significant treatment level, were then compared to initial conditions using Dunnet's test to determine if there was a significant change during the incubation. When significant changes from initial conditions were observed, response variables are discussed as increases or decreases.

Multiple linear regression was used to assess if NDF/CP was the best explanatory variable to explain trends in significant response variables. Other explanatory variables included in these analyses included mg C added, mg N added, and C/N. Since substrates were added on a mass basis, these variables were included to ensure results were not impacted by slight differences in the amount of added C or N. C/N was included in model runs as another potential indicator of substrate quality.

All statistical analyses were conducted using JMP® Pro 11 (SAS Institute Inc., Cary, NC, 1989-2007). Significant results are only discussed using an *a priori* 5% significant level ($\alpha = 0.05$).

3. Results

3.1 Cumulative carbon mineralization and kinetics

Undigested herbage treatments lost more substrate C than ruminal digesta treatments until day 49 (Fig. 2a, SI Table S.3), after which both treatments had similar C remaining at every time point sampled. At the end of the period, ruminal digesta treatments lost similar total soil C as undigested herbage treatments (55.53% and 56.06%

C remaining, respectively) (SI Fig. S.6). The results indicate no impact of substrate quality on bulk soil C loss (and hence stability) after 42 days.

Initial mineralization rates ($C_{\min}$) were 1.2 - 2.1 times higher in soils receiving undigested herbage than those receiving ruminal digesta (Fig. 2b). However, this trend reversed between 21 and 35 d of incubation as ruminal digesta treatments respired 1.1-1.8 times more CO_2 ($\text{mg C kg soil}^{-1} \text{ d}^{-1}$) than the herbage treatments. $C_{\min}$ rates were below $10 \text{ mg C kg soil}^{-1} \text{ d}^{-1}$ for all treatments from 35 d until the end of the experiment.

Two pool decay model fits (SI Table S.4) showed that all undigested herbage treatments had higher active and slow C pool mineralization rate constants than their corresponding undigested herbage treatments ($^{\text{herbage}}k_a = 0.052$ vs. $^{\text{ruminal digesta}}k_a = 0.028$, $^{\text{herbage}}k_s = 0.0014$ vs $^{\text{ruminal digesta}}k_s = 0.0006$, SI Table S.5). Undigested herbage treatments had a smaller active pool sizes, ($C_a = 32.1$ vs. 40.9%), and larger slow pool sizes, ($C_s = 59.1$ vs. 67.9% , SI Table S.5).

3.2 Changes to soil silt C and N pool sizes and isotopic signature

The effect of treatments on silt-associated C during incubation was directly related to the maturity of the substrate (Fig. 3, SI Table S.6). Soils receiving the least mature substrates (14-d) contained more silt C ($23 \text{ g C kg}^{-1} \text{ silt}$) and N ($2.2 \text{ g N kg}^{-1} \text{ silt}$) than those receiving mature substrates (42-d and 35-d), which contained less C (19 and $18 \text{ g C kg}^{-1} \text{ silt}$, respectively) and N (1.8 and $1.7 \text{ g N kg}^{-1} \text{ silt}$, respectively). Soils supplemented with the most mature substrates (42-d) had higher silt $\delta^{13}\text{C}$ values (-20.8%) than all other maturities. No treatments gained or lost significant amounts of

either C or N in this soil fraction compared to the initial soil conditions although the treatments responded differently from one another. In the no-addition controls, the amount and isotopic composition of silt C or silt N did not change over the course of the incubation.

While post-incubation silt C and N pool sizes could best be explained by differences in substrate maturity, silt fraction C/N ratio was best explained by differences in digestion (SI Table S. 6). Soils receiving undigested herbage had a lower C/N ratio (10.3) as compared to those receiving ruminal digesta (10.7).

3.3 Changes to clay C and N pool sizes and isotopic signature

The carbon concentration in the clay fraction was higher at the end of the incubation period for all treatments, collated by digestion or maturity (Fig. 5, Table 2). Similar to the silt fraction, clay fraction C was impacted by the maturity of the substrates (SI Table S.6). Soils receiving 21-d maturity substrates gained more clay C than those receiving 35 and 42-d substrates, resulting in a final concentrations of 106 g C kg⁻¹ clay for the treatments receiving 21-d substrates compared to 91 and 86 g C kg⁻¹ clay, for the treatments receiving 35 and 42-d substrates, respectively. Although soils receiving the 42-d maturity substrates gained less clay C, this C was more enriched in ¹³C (-19.6‰) than C in soils receiving the youngest substrate (-20.1‰) (see SI Fig S.8 for more details). Substrate maturity had no significant effect on clay N. Decomposition of the 21-d substrates increased clay C/N as compared to the 42-d substrates and the initial soil (8.9, 7.1, and 6.6, respectively) (SI Table S.7).

At the termination of the incubation, clay C and N content increased more in soils receiving undigested herbage than in soils receiving ruminal digesta (Fig. 5, SI Table S.6). This resulted in a final C content of 101 g C kg⁻¹ clay and N content of 13 g N kg⁻¹ clay, a significant increase from initial values of 67 g C kg⁻¹ clay and 10 g N kg⁻¹ clay. Clay C increased in soils receiving ruminal digesta, but N content was not significantly altered from the initial soil conditions (Fig. 5, SI Table S.6). Decomposition of native SOM in no addition control treatments resulted in no change in clay fraction C or N pool size as compared to initial conditions, yet enrichment in ¹³C to -20.3‰ at the end of the experiment.

The clay data presented so far was collected on a particle size fraction that likely contained microbial biomass as well as soluble C (Gale and Cambardella, 2000). To estimate the potential contribution from extractable DOC, we rinsed all separated clay fractions with water and measured the C released into solution. The amount of C released during rinsing (0.8-1.8% of clay mass) was not different across treatments and no-addition controls (SI Fig. S.7). However, since soils receiving the ruminal digesta treatments had lower amounts of clay-associated C than the undigested herbage treatments, the relative proportion of clay-C removed during the rinse was higher for the ruminal digesta treatments ($p = 0.0117$, 14.5% of unrinsed clay C). Overall, C removed during the rinse ranged from 6 – 23% of unrinsed clay C. Re-calculating the clay-associated C by adjusting for C removed during the rinse (Clay_r-C) did not change the pattern of any of our results (Table 2, SI Table S.6). Similar to our initial findings, we found ruminal digesta treatments accumulated less clay_r-C than the undigested herbage treatments resulting in a final value of 76 g C kg⁻¹ clay as compared to 90 g C kg⁻¹ clay.

As in the previous clay C estimate, more C accumulated after incubation of the 21-d maturity substrates (93 g C kg⁻¹ clay) than after incubation of the 42-d maturity substrates (73 g C kg⁻¹ clay).

3.4 Relationships between substrate quality, mineralization kinetics, and soil fraction C and N pools

Multiple regression analyses, with amount of substrate C added (mg), amount of substrate N added (mg), and substrate C/N ratio included along with NDF/CP, indicated that NDF/CP was the best explanatory variable (SI Table S.7). The kinetics of soil C mineralization as well as the final silt and clay C content were inversely correlated with our substrate quality proxy (NDF/CP) and thus directly correlated with substrate quality (i.e., higher quality ~ higher mineralization rates and C content). The rate of SOC mineralization (active and slow pool rate constant, k_a , k_s) increased as our index for substrate quality increased (i.e., as NDF/CP decreased) (Fig 4). NDF/CP was also negatively correlated with final silt fraction C and N ($p = 0.0006$, data not shown), as well as changes to unrinsed clay C and clay_r-C (Fig 5). Changes in clay-N followed a similar trend, although this was not significant ($p = 0.064$).

4. Discussion

Microbial biomass is recognized as an important precursor to the formation of stable SOM (Grandy and Neff, 2008; Miltner et al., 2012; Hobara et al., 2014;

Kallenbach et al., 2015). Our study illustrates a relationship between the quality of carbon inputs and the quantity of silt and clay fraction SOM formed during processing by the soil microbial community. These observations lend support the MEMS hypothesis that labile substrates are the “main precursors of stable C” (Cotrufo et al., 2013). Although we cannot estimate the long-term stability (i.e., millennial scale) of the newly accumulated clay C (Clay_r-C), we do show that it is biologically stable under short-term (<180 days), non-limiting temperature and moisture conditions.

4.1 Substrate quality influences bulk soil mineralization dynamics

Soils receiving higher quality, undigested herbage substrates initially produced CO₂ at a faster rate than soils receiving lower quality, ruminal digesta substrates. The direct relationship of substrate quality on decomposition kinetics is evident in the initial rates of CO₂ production (Fig 2, Fig 5.). Mineralization rate constants are generally lower than those reported elsewhere because we included soil carbon in the active pool, whereas most other studies determine rate constants based on substrate C remaining. For instance, we found k_a values between 0.020 – 0.056 d⁻¹ (SI Table S.5), Sanaullah et al (2010) found k_a values of 0.15 – 0.47 d⁻¹ when assessing the decomposition of three C3 grass species in the field and Johnson et al (2007) found k_a values of 0.032 – 0.18 d⁻¹ across a wider range of crop litter types in laboratory incubations. Fitting the model using the same methodology as these authors (data not shown), produced k_a values for the undigested bermudagrass litter (0.09 – 0.12 d⁻¹) that were similar to those found for other C4 grass leaf and stem litter (corn and switchgrass, 0.06 – 0.09 d⁻¹) under similar

laboratory conditions (Johnson et al., 2007). Sanaullah et al. (2010) generally observed higher active pool sizes and k_a values for green litter compared to brown (dead) litter, similar to the trends we observed between undigested and digested bermudagrass in our initial model.

Ultimately, in our case, we fit our rate constants in terms of total soil C (inputs C + native soil C) and not solely in terms of input C (determined by subtracting no-addition control respiration) since we had strong evidence that mineralization of pre-existing SOM differed between addition and no-addition controls (Section 2.5). Despite differences in methodology, we observe a similar negative correlation between substrate quality (NDF/CP) and k_a and k_s (Fig. 4) as other studies. Positive correlations of k_a and k_s with initial tissue %N as well as a negative correlation of k_a with tissue cellulose, hemicellulose, and lignin content have been observed for leaf, stem, and root crop biomass (Johnson et al., 2007), agreeing with our observations. Walela et al., 2014 found similar negative correlations of k_a and k_s with initial tissue lignin, lignin/N, as well as lignin+tannin/N. They found no relationship between rate constants and cellulose or cellulose/N ratio, indicating that the relationship observed in our study may be strongly driven by lignin content (as reflected in the NDF measurements). Ruminant digestion is the main driver of both regressions (Fig 4, SI Table S.5) suggesting that other parameters not captured by this quality index may be responsible as well. It is important to note that these observations generally align with the negative correlation between decomposition rate and lignin/N evident from litterbag decomposition studies (Zhang et al. 2008; Prescott, 2010).

Bulk soil carbon content is not a sensitive indicator of short-term changes in C dynamics (Neff et al., 2002). If only bulk C is monitored often the impacts of litter quality or mineralogical influence remain “hidden” (Vogel et al., 2015). In line with this view, substrate quality did not impact the cumulative CO₂ produced during our incubation nor did it impact the bulk SOC pool sizes (Table 2, SI Table S.6). Stewart et al (2015) found that even when CO₂ production followed different kinetic patterns due to substrate quality (lignin content), cumulative respired C was similar. Our results are consistent with the reactivity-stabilization hypothesis (Yang and Janssen, 2002) in that herbage lost more C than the ruminal digesta treatments initially (Fig. 2), but by 139 days all treatments converged to have similar amounts of cumulative C loss (SI Fig. S.6). Observations of high quality substrates leading to more bulk SOC have been observed under longer-term experimental conditions (7 – 42 years) when looking at manipulated substrate additions (Voroney et al., 1989, Kirchmann et al., 2004) or using a common garden approach (Mueller et al., 2015).

4.2 Formation of silt and clay associated SOC influenced by quality of inputs

In this study, we document a clear role of initial substrate quality on the amount of silt and clay sized SOC (Fig. 5). SOM in these particle-size fractions (especially the fine soil particles, <20 µm) are often considered more resistant to microbial decomposition than the bulk soil and associated with mineral phases (Hassink, 1997). Carbon in the soil silt and clay fractions tends to turnover slower and have older average ¹⁴C ages (Balesdent et al., 1998; von Lützow, 2006; Koegel Knabner et al., 2008)—

though portions of this C (and N) can be desorbed and mineralized (Jones et al., 1998; Mikutta et al., 2007, Bird et al., 2008), and therefore will turnover at faster rates (Kleber et al., 2007). While there is likely some clay-sized particulate OM in the clay fraction, we believe this fractionation scheme isolates a predominantly mineral-associated fraction of SOM as effectively as density fractionation or a combined approach (Diochon et al., 2016).

Our results agree with Cotrufo et al.'s (2015) observations that the decomposition of labile substrates is the primary step leading to the formation of mineral-associated SOM during the early stages of decomposition. As work with artificial soils has indicated (Vogel et al., 2015), we observe that higher initial mineralization rates (larger k_a) result in the formation of more C in the silt and clay fraction (Fig. 4, 5). Within this paradigm, higher quality substrates are likely to lead to more mineral-associated C because they are metabolized faster and more efficiently than low quality substrates. The relationship between first order decay rates and efficient microbial C-use may only apply for more complex substrates (Liang et al., 2007, Bird et al., 2008), whereas decay rates determined by measurements of CO₂ may show the opposite trend for low molecular weight (LMW) substances (Bradford et al., 2013). This is likely due to the fact that initial CO₂ measurements signals are dominated by the rapid uptake of higher amounts of soluble components for complex resources, while they more directly represent the inefficient use of C (metabolic pathway discrimination) of LMW substrates that are all rapidly assimilated (Gunina et al., 2014).

Field-scale research involving applications of differing quality residues provides evidence supporting our observations of increased silt and clay C under higher quality

treatments (Fig 5). Long-term manure applications generated more silt and clay associated C than applications of a lower quality (higher C:N) peat applications (Kirchmann et al., 2004; Gerzabek et al., 2006). Similarly, adding N—irrespective of the source—can increase mineral-associated C and N (< 5 μm) presumably because it increases the quality of existing substrates and increases microbial C cycling (Gillespie et al., 2014).

However, in some cases litter quality does not appear to influence SOM. For instance, Helfrich et al. (2008) found litter quality did not impact bulk or mineral associated SOM content (litter quality only impacted soil aggregate formation). Other studies suggest mineral composition alone—and not substrate chemistry—drives mineral stabilization of C inputs (Throckmorton et al., 2012; Throckmorton et al., 2015).

Castellano et al. (2015) have posited that mineral-stabilization of input derived C and N is only impacted by substrate quality when soil C concentrations are below a theoretical saturation capacity based on the mineral surface area (Stewart et al., 2007; Six et al., 2002). The soils in our study were depleted in C from previous land use history and capable of accumulating bulk C at rates as high as $8 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ (Machmuller et al., 2015). We do not know the carbon stabilization capacity for this soil precisely, but we estimate it is 4 – 10 times higher than initial conditions [see SI Table S.8 and accompanying text for calculations based on several predictive models]. Our estimate also illustrates the initial C concentration in the clay fraction (Table 2) is considerably lower than most soil clay fraction C concentrations: for instance, our soil's clay fraction C concentrations is at least an order of magnitude lower than the median reported for 213 soils from New Zealand (Beare et al., 2014) and it is nearly half of the values reported for

the fine fraction C in Dutch grassland horizons (Hassink et al., 1997). The initial C-deficiency of our soil clay fraction likely explains why C accumulates following substrate addition; where as other soils near their C-saturation do not (Castellano et al., 2015, citations therein). Consistent with our results, Puttaso et al. (2013) found sandy soils (which presumably also had C-deficient clay fractions) accumulated C in the fine mineral fraction and that the quality of litter inputs influenced the magnitude of C accumulation.

The clay fraction of our soils contains hydroxy-interlayered vermiculite (HIV), kaolinite, and Fe^{III} oxyhydroxide phases. All of these mineral phases offer potential surfaces for C stabilization (SI Fig S.1, S.5, Table S.2), yet the short-range-ordered Fe-oxyhydroxide phases are likely the dominant mineral phase involved in this process (Kramer et al., 2012). Fe^{III} oxyhydroxides have much higher specific surface area (SSA) and tend to dominate the total measured SSAs in clay fractions extracted from surficial horizons (Eusterhues et al., 2005). Short range ordered Fe^{III} oxyhydroxide phases represent the majority of iron in our sample (Table S.2), with over 50% of the iron present of a lower crystallinity than typical laboratory-prepared ferrihydrite (Fig S.5, Table S.2), which magnetically orders at 77K in a Mössbauer spectra. To evaluate the localization of incubation-resistant organic matter in this system, a more substantially isotopically enriched substrate could be probed using nanoscale secondary ion mass spectrometry methods (Keiluweit et al., 2012, Vogel et al., 2014).

5. Conclusions

Our work presents empirical evidence that during the decomposition of complex residues in soils, more labile substrates lead to a greater persistence of SOM in the soil silt and clay fraction than less labile substrates. These findings support the MEMS framework (Cotrufo et al., 2013) and emphasize the emerging concept that lower C mineralization rates do not necessarily translate into more “stable” carbon. The fact that such minor differences in substrate quality could significantly impact clay fraction C and N highlights the need for a better empirical understanding of conditions under which substrate quality, microbial substrate use efficiency, and mineralogical characteristics interact to determine the SOM loadings of silt and clay mineral fractions.

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References

- Angers, D.A., Recous, S., Aita, C., 1997. Fate of carbon and nitrogen in water-stable aggregates during decomposition of (CN)-C-13-N-15-labelled wheat straw in situ. *European Journal of Soil Science* 48, 295-300.
- Balesdent, J., Besnard, E., Arrouays, D., Chenu, C., 1998. The dynamics of carbon in particle-size fractions of soil in a forest-cultivation sequence. *Plant and Soil* 201, 49-57.
- Beardmore, R.E., Gudelj, I., Lipson, D.A., Hurst, L.D., 2011. Metabolic trade-offs and the maintenance of the fittest and the flattest. *Nature* 472, 342-346.
- Beare, M., McNeill, S., Curtin, D., Parfitt, R., Jones, H., Dodd, M., Sharp, J., 2014. Estimating the organic carbon stabilisation capacity and saturation deficit of soils: a New Zealand case study. *Biogeochemistry*, 1-17.
- Bird, J.A., Kleber, M., Torn, M.S., 2008. C-13 and N-15 stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. *Organic Geochemistry* 39, 465-477.
- Bradford, M.A., Keiser, A.D., Davies, C.A., Mersmann, C.A., Strickland, M.S., 2013. Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. *Biogeochemistry* 113, 271-281.
- Castellano, M.J., Mueller, K.E., Olk, D.C., Sawyer, J.E., Six, J., 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. *Global Change Biology* 21, 3200-3209.
- Chadwick, D.R., John, F., Pain, B.F., Chambers, B.J., Williams, J., 2000. Plant uptake of nitrogen from the organic nitrogen fraction of animal manures: a laboratory experiment. *Journal of Agricultural Science* 134, 159-168.
- Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology* 19, 988-995.
- Diochon, A., Gillespie, A.W., Ellert, B.H., Janzen, H.H., Gregorich, E.G., 2016. Recovery and dynamics of decomposing plant residue in soil: an evaluation of three fractionation methods. *European Journal of Soil Science* 67, 196-205.
- Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biology* 18, 1781-1796.
- Eusterhues, K., Rumpel, C., Kögel-Knabner, I., 2005. Organo-mineral associations in sandy acid forest soils: importance of specific surface area, iron oxides and micropores. *European Journal of Soil Science* 56, 753-763.
- Feng, W.T., Plante, A.F., Six, J., 2013. Improving estimates of maximal organic carbon stabilization by fine soil particles. *Biogeochemistry* 112, 81-93.
- Gale, W.J., Cambardella, C.A., 2000. Carbon dynamics of surface residue- and root-derived organic matter under simulated no-till. *Soil Science Society of America Journal* 64, 190-195.
- Gentile, R., Vanlauwe, B., Six, J., 2011. Litter quality impacts short- but not long-term soil carbon dynamics in soil aggregate fractions. *Ecological Applications* 21, 695-703.

- Gerzabek, M.H., Antil, R.S., Kögel-Knabner, I., Knicker, H., Kirchmann, H.,
Haberhauer, G., 2006. How are soil use and management reflected by soil organic
matter characteristics: a spectroscopic approach. *European Journal of Soil Science*
57, 485-494.
- Grandy, A.S., Neff, J., 2008. Molecular C dynamics downstream: the biochemical
decomposition sequence and its impact on soil organic matter structure and
function. *Science of the Total Environment* 404, 297-307.
- Gunina, A., Dippold, M.A., Glaser, B., Kuzyakov, Y., 2014. Fate of low molecular
weight organic substances in an arable soil: From microbial uptake to utilisation
and stabilisation. *Soil Biology and Biochemistry* 77, 304-313.
- Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association
with clay and silt particles. *Plant and Soil* 191, 77-87.
- Hatton, P.J., Castanha, C., Torn, M.S., Bird, J.A., 2015. Litter type control on soil C and
N stabilization dynamics in a temperate forest. *Global Change Biology* 21, 1358-
1367.
- Helfrich, M., Ludwig, B., Potthoff, M., Flessa, H., 2008. Effect of litter quality and soil
fungi on macroaggregate dynamics and associated partitioning of litter carbon and
nitrogen. *Soil Biology & Biochemistry* 40, 1823-1835.
- Hill, N.S., Cabrera, M.L., Agee, C.S., 1995. Morphological and climatological predictors
of forage quality in tall fescue. *Crop Science* 35, 541-549.
- Hobara, S., Osono, T., Hirose, D., Noro, K., Hirota, M., Benner, R., 2014. The roles of
microorganisms in litter decomposition and soil formation. *Biogeochemistry* 118,
471-486.
- Hobbie, S.E., 2005. Contrasting effects of substrate and fertilizer nitrogen on the early
stages of litter decomposition. *Ecosystems* 8, 644-656.
- Horwitz, W., 2000. Official methods of analysis of AOAC International. AOAC
International, Gaithersburg, Md.
- Janzen, H.H., 2006. The soil carbon dilemma: Shall we hoard it or use it? *Soil Biology &
Biochemistry* 38, 419-424.
- Jenkins, T.C., Bridges, W.C., Harrison, J.H., Young, K.M., 2014. Addition of potassium
carbonate to continuous cultures of mixed ruminal bacteria shifts volatile fatty
acids and daily production of biohydrogenation intermediates. *Journal of dairy
science* 97, 975-984.
- Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and
its relation to climate and vegetation. *Ecological Applications* 10, 423-436.
- Johnson, J.M.F., Barbour, N.W., Weyers, S.L., 2007. Chemical composition of crop
biomass impacts its decomposition. *Soil Science Society of America Journal* 71,
155-162.
- Jones, D.L., Edwards, A.C., 1998. Influence of sorption on the biological utilization of
two simple carbon substrates. *Soil Biology & Biochemistry* 30, 1895-1902.
- Jost, D.I., Joergensen, R.G., Sundrum, A., 2013. Effect of cattle faeces with different
microbial biomass content on soil properties, gaseous emissions and plant growth.
Biology and Fertility of Soils 49, 61-70.
- Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial
physiology and necromass regulate agricultural soil carbon accumulation. *Soil
Biology & Biochemistry* 91, 279-290.

- Kalra, Y.P., 1995. Determination of pH of soils by different methods: collaborative study. *Journal of AOAC International* 78, 310-324.
- Keiluweit, M., Bougoure, J.J., Zeglin, L.H., Myrold, D.D., Weber, P.K., Pett-Ridge, J., Kleber, M., Nico, P.S., 2012. Nano-scale investigation of the association of microbial nitrogen residues with iron (hydr) oxides in a forest soil O-horizon. *Geochimica Et Cosmochimica Acta* 95, 213-226.
- Kettler, T.A., Doran, J.W., Gilbert, T.L., 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. *Soil Science Society of America Journal* 65, 849-852.
- Kirchmann, H., Haberhauer, G., Kandeler, E., Sessitsch, A., Gerzabek, M.H., 2004. Effects of level and quality of organic matter input on carbon storage and biological activity in soil: Synthesis of a long-term experiment. *Global Biogeochemical Cycles* 18, 9.
- Kirkby, C.A., Richardson, A.E., Wade, L.J., Batten, G.D., Blanchard, C., Kirkegaard, J.A., 2013. Carbon-nutrient stoichiometry to increase soil carbon sequestration. *Soil Biology & Biochemistry* 60, 77-86.
- Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Chapter One - Mineral–Organic Associations: Formation, Properties, and Relevance in Soil Environments, In: Donald, L.S. (Ed.), *Advances in Agronomy*. Academic Press, pp. 1-140.
- Kleber, M., Johnson, M.G., 2010. Chapter 3 - Advances in Understanding the Molecular Structure of Soil Organic Matter: Implications for Interactions in the Environment, In: Donald, L.S. (Ed.), *Advances in Agronomy*. Academic Press, pp. 77-142.
- Kleber, M., Nico, P., Plante, A., Filley, T., Kramer, M., 2011. Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. *Global Change Biology* 17, 1097-1107.
- Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry* 85, 9-24.
- Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., 2008. Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science* 171, 61-82.
- Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry* 34, 139-162.
- Kramer, M.G., Sander, J., Chadwick, O.A., Chorover, J., Vitousek, P.M., 2012. Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. *Global Change Biology* 18, 2594-2605.
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature* 528, 60-68.
- Lekkerkerk, L., Lundkvist, H., Agren, G.I., Ekbohm, G., Bosatta, E., 1990. Decomposition of heterogeneous substrates - an experimental investigation of a hypothesis on substrate and microbial properties. *Soil Biology & Biochemistry* 22, 161-167.

704 Liang, C., Zhang, X., Balser, T.C., 2007. Net microbial amino sugar accumulation
 705 process in soil as influenced by different plant material inputs. *Biology and*
 706 *Fertility of Soils* 44, 1-7.
 707 Machmuller, M.B., Kramer, M.G., Cyle, T.K., Hill, N., Hancock, D., Thompson, A.,
 708 2015. Emerging land use practices rapidly increase soil organic matter. *Nat*
 709 *Commun* 6.
 710 Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: Theory and
 711 models across scales. *Soil Biology & Biochemistry* 41, 1355-1379.
 712 Manzoni, S., Taylor, P., Richter, A., Porporato, A., Agren, G.I., 2012. Environmental and
 713 stoichiometric controls on microbial carbon-use efficiency in soils. *New*
 714 *Phytologist* 196, 79-91.
 715 Meentemeyer, V., 1978. Macroclimate and lignin control of litter decomposition rates.
 716 *Ecology* 59, 465-472.
 717 Mikutta, R., Kleber, M., Torn, M.S., Jahn, R., 2006. Stabilization of soil organic matter:
 718 Association with minerals or chemical recalcitrance? *Biogeochemistry* 77, 25-56.
 719 Miltner, A., Bombach, P., Schmidt-Brucken, B., Kastner, M., 2012. SOM genesis:
 720 microbial biomass as a significant source. *Biogeochemistry* 111, 41-55.
 721 Moore, D.M., Reynolds, R.C., 1997. X-ray Diffraction and the Identification and
 722 Analysis of Clay Minerals. Oxford University Press.
 723 Mueller, K.E., Hobbie, S.E., Chorover, J., Reich, P.B., Eisenhauer, N., Castellano, M.J.,
 724 Chadwick, O.A., Dobies, T., Hale, C.M., Jagodzinski, A.M., Kalucka, I.,
 725 Kieliszewska-Rokicka, B., Modrzynski, J., Rozen, A., Skorupski, M., Sobczyk,
 726 L., Stasinska, M., Trocha, L.K., Weiner, J., Wierzbicka, A., Oleksyn, J., 2015.
 727 Effects of litter traits, soil biota, and soil chemistry on soil carbon stocks at a
 728 common garden with 14 tree species. *Biogeochemistry* 123, 313-327.
 729 Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D.,
 730 2002. Variable effects of nitrogen additions on the stability and turnover of soil
 731 carbon. *Nature* 419, 915-917.
 732 Pal, P., Clough, T.J., Kelliher, F.M., van Koten, C., Sherlock, R.R., 2012. Intensive Cattle
 733 Grazing Affects Pasture Litter-Fall: An Unrecognized Nitrous Oxide Source.
 734 *Journal of Environmental Quality* 41, 444-448.
 735 Paul, E.A., Morris, S.L., Bohm, S., 2001. The determination of soil C pool sizes and
 736 turnover rates: Tracers and biophysical fractionation., In: Lal, R. (Ed.),
 737 Assessment methods for soil carbon. Lewis Publ., Boca Raton, FL, pp. 193-206.
 738 Prescott, C., 2010. Litter decomposition: what controls it and how can we alter it to
 739 sequester more carbon in forest soils? *Biogeochemistry* 101, 133-149.
 740 Puttaso, A., Vityakon, P., Rasche, F., Saenjan, P., Treloges, V., Cadisch, G., 2013. Does
 741 Organic Residue Quality Influence Carbon Retention in a Tropical Sandy Soil?
 742 *Soil Science Society of America Journal* 77, 1001-1011.
 743 Rubino, M., Dungait, J.A.J., Evershed, R.P., Bertolini, T., De Angelis, P., D'Onofrio, A.,
 744 Lagomarsino, A., Lubritto, C., Merola, A., Terrasi, F., Cotrufo, M.F., 2010.
 745 Carbon input belowground is the major C flux contributing to leaf litter mass loss:
 746 Evidences from a C-13 labelled-leaf litter experiment. *Soil Biology &*
 747 *Biochemistry* 42, 1009-1016.

- Rubino, M., Lubritto, C., D'Onofrio, A., Terrasi, F., Gleixner, G., Cotrufo, M.F., 2007. An isotopic method for testing the influence of leaf litter quality on carbon fluxes during decomposition. *Oecologia* 154, 155-166.
- Sanaullah, M., Chabbi, A., Lemaire, G., Charrier, X., Rumpel, C., 2010. How does plant leaf senescence of grassland species influence decomposition kinetics and litter compounds dynamics? *Nutrient Cycling in Agroecosystems* 88, 159-171.
- Schlesinger, W.H., Bernhardt, E.S., 2013. Chapter 5 - The Biosphere: The Carbon Cycle of Terrestrial Ecosystems, In: Bernhardt, W.H.S.S. (Ed.), *Biogeochemistry* (Third Edition). Academic Press, Boston, pp. 135-172.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49-56.
- Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil* 241, 155-176.
- Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2007. Soil carbon saturation: concept, evidence and evaluation. *Biogeochemistry* 86, 19-31.
- Thiet, R.K., Frey, S.D., Six, J., 2006. Do growth yield efficiencies differ between soil microbial communities differing in fungal: bacterial ratios? Reality check and methodological issues. *Soil Biology & Biochemistry* 38, 837-844.
- Throckmorton, H.M., Bird, J.A., Dane, L., Firestone, M.K., Horwath, W.R., 2012. The source of microbial C has little impact on soil organic matter stabilisation in forest ecosystems. *Ecology Letters* 15, 1257-1265.
- Throckmorton, H.M., Bird, J.A., Monte, N., Doane, T., Firestone, M.K., Horwath, W.R., 2015. The soil matrix increases microbial C stabilization in temperate and tropical forest soils. *Biogeochemistry* 122, 35-45.
- Tiessen, H., Stewart, J.W.B., Hunt, H.W., 1984. Concepts of soil organic-matter transformations in relation to organo-mineral particle-size fractions. *Plant and Soil* 76, 287-295.
- Van Soest, P.v., Robertson, J., Lewis, B., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of dairy science* 74, 3583-3597.
- van Vliet, P.C.J., Reijs, J.W., Bloem, J., Dijkstra, J., de Goede, R.G.M., 2007. Effects of cow diet on the microbial community and organic matter and nitrogen content of feces. *Journal of dairy science* 90, 5146-5158.
- Vogel, C., Mueller, C.W., Hoschen, C., Buegger, F., Heister, K., Schulz, S., Schlöter, M., Kogel-Knabner, I., 2014. Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. *Nature Communications* 5, 7.
- Vogel, C., Heister, K., Buegger, F., Tanuwidjaja, I., Haug, S., Schlöter, M., Kögel-Knabner, I., 2015. Clay mineral composition modifies decomposition and sequestration of organic carbon and nitrogen in fine soil fractions. *Biology and Fertility of Soils* 51, 427-442.
- von Lützow, M., Kogel Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., 2006. Stabilization of organic matter in temperate soils: mechanisms and their

- relevance under different soil conditions - a review. *European Journal of Soil Science* 57, 426-445.
- von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry* 39, 2183-2207.
- Voroney, R.P., Paul, E.A., Anderson, D.W., 1989. Decomposition of wheat straw and stabilization of microbial products. *Canadian journal of soil science* 69, 63-77.
- Waksman, S.A., Tenney, F.G., 1927. The composition of natural organic materials and their decomposition in the soil II Influence of age of plant upon the rapidity and nature of its decomposition - Rye plants. *Soil Science* 24, 317-333.
- Walela, C., Daniel, H., Wilson, B., Lockwood, P., Cowie, A., Harden, S., 2014. The initial lignin:nitrogen ratio of litter from above and below ground sources strongly and negatively influenced decay rates of slowly decomposing litter carbon pools. *Soil Biology & Biochemistry* 77, 268-275.
- Wieder, W.R., Bonan, G.B., Allison, S.D., 2013. Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change* 3, 909-912.
- Wieder, W.R., Grandy, A.S., Kallenbach, C.M., Taylor, P.G., Bonan, G.B., 2015. Representing life in the Earth system with soil microbial functional traits in the MIMICS model. *Geoscientific Model Development* 8, 1789-1808.
- Williams, M.A., Myrold, D.D., Bottomley, P.J., 2006. Carbon flow from C-13-labeled straw and root residues into the phospholipid fatty acids of a soil microbial community under field conditions. *Soil Biology & Biochemistry* 38, 759-768.
- Yang, H.S., Janssen, B.H., 2002. Relationship between substrate initial reactivity and residues ageing speed in carbon mineralization. *Plant and Soil* 239, 215-224.
- Yeasmin, S., Singh, B., Kookana, R.S., Farrell, M., Sparks, D.L., Johnston, C.T., 2014. Influence of mineral characteristics on the retention of low molecular weight organic compounds: A batch sorption-desorption and ATR-FTIR study. *Journal of Colloid and Interface Science* 432, 246-257.
- Zhang, D., Hui, D., Luo, Y., Zhou, G., 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of plant ecology* 1, 85-93.
- Zibilske, L. M., 1994. Carbon mineralization, in: *Methods of Soil Analysis. Part 2. Microbiological* 683 and biochemical properties, SSSA Book Series 5, edited by: Weaver, R. W., Angel, J. S. and 684 Bottomley, P. S., Madison, Wisconsin, 835-863.

Table 1 Substrate Characteristics

		%C	δ ¹³ C	%N	C/N	%NDF	%CP	NDF/CP
Type	Maturity							
Undigested Herbage	14	43.93	-16.28	3.06	14.4	52	21.2	2.45
Undigested Herbage	21	44.16	-15.11	2.9	15.2	56.8	19.0	2.99
Undigested Herbage	28	43.59	-14.5	2.42	18.0	62.1	16.1	3.86
Undigested Herbage	35	43.70	-13.89	2.3	19.0	60.5	15.4	3.93
Undigested Herbage	42	42.84	-7.81	3.02	14.2	63.1	15.3	4.12
Ruminal Digesta	14	44.42	-17.51	4.27	10.4	57.5	13.9	4.14
Ruminal Digesta	21	43.50	-16.6	3.62	12.0	60	12.7	4.72
Ruminal Digesta	28	43.02	-15.27	3.32	13.0	67.6	10.8	6.26
Ruminal Digesta	35	43.16	-14.39	2.91	14.8	70.3	10.0	7.03
Ruminal Digesta	42	43.05	-15.36	3.06	14.1	68.7	10.3	6.67

CP-crude protein
NDF-neutral detergent fiber

Table 2 Soil Particle Size Fraction C and N over 139 d incubation

Soil Treatments		Bulk Fraction*					Silt Sized (53 µm-2 µm)					Clay Sized (< 2 µm)					Clay< (< 2 µm)	
		C (g kg ⁻¹ soil)	δ ¹³ C	N (g kg ⁻¹ soil)	δ ¹⁵ N	C/N	C (g kg ⁻¹ silt)	δ ¹³ C	N (g kg ⁻¹ silt)	δ ¹⁵ N	C/N	C (g kg ⁻¹ clay)	δ ¹³ C	N (g kg ⁻¹ clay)	δ ¹⁵ N	C/N	C (g kg ⁻¹ clay)	C (g kg ⁻¹ soil)
Initial Soil Conditions		6.6 (0.1)	-20.32 (0.11)	0.06 (0.00)	5.71 (0.11)	11.14 (0.13)	20.3 (1.4)	-21.27 (0.05)	2.0 (0.1)	6.38 (0.09)	10.27 (0.11)	67.4 (0.9)	-20.96 (0.03)	10.3 (0.1)	7.41 (0.07)	6.58 (0.07)	53.5 (0.5)	1.42
Substrate Treatments at 139 d																		
Type	Maturity (d)																	
Undigested Herbage	14	6.5 (0.7)	-20.11 (0.20)	0.7 (0.1)	5.96 (0.44)	9.18 (0.38)	23.0 (1.8)	-21.15 (0.08)	2.3 (0.2)	6.68 (0.31)	10.05 (0.10)	107.8 (2.3)	-20.02 (0.06)	14.1 (0.8)	9.16 (0.89)	7.73 (0.31)	98.33 (2.5)	2.65
Undigested Herbage	21	5.8 (0.2)	-19.93 (0.27)	0.6 (0.0)	6.06 (0.36)	10.07 (0.13)	21.2 (1.3)	-20.89 (0.18)	2.1 (0.1)	6.90 (0.25)	10.35 (0.17)	106.6 (8.2)	-19.73 (0.24)	12.5 (0.2)	9.19 (0.72)	8.55 (0.69)	95.1 (1.9)	2.57
Undigested Herbage	28	7.4 (0.7)	-19.88 (0.24)	0.8 (0.1)	5.92 (0.26)	9.522 (0.37)	23.5 (0.9)	-20.98 (0.12)	2.3 (0.1)	6.57 (0.06)	10.44 (0.31)	103.4 (2.5)	-19.8 (0.17)	13.8 (1.1)	8.98 (0.58)	7.67 (0.60)	92.9 (3.0)	2.51
Undigested Herbage	35	7.1 (0.6)	-19.86 (0.15)	0.7 (0.1)	6.07 (0.36)	9.77 (0.36)	20.1 (1.0)	-20.97 (0.05)	1.9 (0.1)	6.76 (0.32)	10.40 (0.18)	98.6 (6.9)	-19.81 (0.08)	12.1 (0.4)	8.86 (0.81)	8.17 (0.57)	87.8 (6.8)	2.37
Undigested Herbage	42	7.4 (0.3)	-19.66 (0.16)	0.8 (0.0)	6.24 (0.17)	9.15 (0.20)	20.1 (0.7)	-20.71 (0.06)	1.9 (0.1)	6.71 (0.16)	10.45 (0.12)	87.8 (3.1)	-19.20 (0.06)	13.2 (0.8)	9.01 (0.37)	6.70 (0.31)	76.4 (3.8)	2.06
Ruminal Digesta	14	6.7 (0.5)	-20.09 (0.15)	0.7 (0.0)	6.34 (0.79)	9.56 (0.40)	22.6 (0.8)	-21.42 (0.06)	2.1 (0.1)	6.82 (0.50)	10.65 (0.36)	87.0 (2.7)	-20.29 (0.04)	10.8 (0.7)	9.33 (1.54)	8.21 (0.88)	74.4 (1.9)	2.01
Ruminal Digesta	21	6.9 (0.7)	-19.92 (0.15)	0.7 (0.1)	5.1 (0.51)	10.03 (0.12)	18.1 (1.4)	-21.47 (0.09)	1.7 (0.1)	6.31 (0.25)	10.73 (0.07)	105 (5.1)	-19.84 (0.18)	11.3 (0.3)	7.50 (1.04)	9.31 (0.31)	91.5 (5.2)	2.47
Ruminal Digesta	28	5.8 (0.6)	-20.17 (0.12)	0.6 (0.1)	5.71 (0.65)	9.98 (0.13)	16.1 (0.7)	-21.67 (0.05)	1.5 (0.1)	6.90 (0.46)	11.07 (0.16)	85.9 (5.5)	-20.06 (0.10)	10.2 (0.2)	9.13 (1.51)	8.37 (0.40)	72.6 (5.5)	1.96
Ruminal Digesta	35	5.3 (0.7)	-19.95 (0.08)	0.6 (0.1)	5.75 (0.38)	8.93 (0.24)	15.5 (1.4)	-21.22 (0.18)	1.5 (0.1)	6.65 (0.18)	10.62 (0.11)	82.6 (3.9)	-19.87 (0.03)	12.1 (0.7)	8.74 (0.76)	6.98 (0.67)	71.8 (3.4)	1.94
Ruminal Digesta	42	6.3 (0.1)	-20.08 (0.20)	0.6 (0.1)	4.97 (0.30)	9.98 (0.35)	17.0 (2.0)	-20.95 (0.14)	1.6 (0.2)	6.34 (0.09)	10.71 (0.21)	83.3 (5.0)	-19.91 (0.11)	11.4 (0.8)	7.19 (0.41)	7.46 (0.62)	69.3 (5.5)	1.87
No Addition Control at 139 d		7.4 (0.5)	-20.28 (0.21)	0.7 (0.0)	6.432 (0.13)	10.99 (0.25)	20.3 (1.4)	-21.21 (0.09)	2.0 (0.1)	6.21 (0.23)	10.36 (0.36)	62.3 (1.7)	-20.33 (0.07)	10.9 (0.6)	9.26 (0.39)	5.74 (0.18)	51.7 (2.0)	1.40

Means are depicted with accompanying standard error (n=5), except for Ruminal Digesta 14-d and 28-d (n=3)

*High standard errors associated with bulk measurements are likely due to small sampling mass (~2 g equivalent dry mass), while particle size fractions were separated from ~25 g equivalent dry mass.

Fig. 1. Neutral detergent fiber (NDF) to crude protein content (CP) ratios across all factorial treatment combinations.

Fig. 2. Soil Carbon mineralization dynamics. Percent of soil carbon remaining ($C_{\text{remaining}}$) for treatments collated by digestion over the first 49 days of the incubation (a) and Substrate carbon mineralization rates (C_{min}) for treatments collated by digestion throughout the 139 d incubation (b). All maturities were combined as only digestion, time, and digestion*time were found to be statistically significant (SI Table S.3, $\alpha = 0.05$). Mean $C_{\text{remaining}}$ values for each treatment depicted are statistically different (Student's t-test, $\alpha = 0.05$) until the 42-49 day interval. Error bars represent standard error. Asterisks located on Panel B indicate intervals where digestion where calculated rates were statistically different (Student's t-test, $\alpha = 0.05$).

Fig. 3. Carbon and nitrogen fractions in silt and clay-sized fractions post incubation. Error bars represent standard error. For each panel, data points have been collated by significant treatment effects (SI Table. S.3, $\alpha = 0.05$). $\delta^{15}N$ was not a significant response variable within the fully factorial model. While not included in the statistical model, no addition controls and initial soil conditions have been included as a reference point. (UH-undigested herbage, RD-ruminal digesta, NAC-no addition control, IC-initial soil conditions).

Fig 4. Regression correlations of estimated k_a (circles) and k_s (triangles) from a two-pool decay model. Estimated means are visually collated by digestion (unfilled - undigested herbage, filled - ruminal digesta).

Fig 5. Regression correlations of estimated k_a as well as silt and rinsed clay (Clay_r) fraction C data with added substrate quality, as assessed by NDF/CP. Error bars represent standard error.