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Substrate quality and concentration control decomposition and microbial strategies in a model soil system

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Abstract Soil carbon models typically scale decomposition linearly with soil carbon (C) concentration, but this linear relationship has not been experimentally verified. Here we investigated the underlying biogeochemical mechanisms controlling the relationships between soil C concentration and decomposition rates. We incubated a soil/sand mixture with increasing amounts of finely ground plant residue in the laboratory at constant temperature and moisture for 63 days. The plant residues were rye (*Secale cereale*, C/N ratio of 23) and wheat straw (*Triticum* spp., C/N ratio of 109) at seven soil C concentrations ranging from 0.38 to 2.99%. We measured soil respiration, dissolved organic carbon (DOC) concentrations, microbial biomass, and potential enzyme activities over the course of the incubation. Rye, which had higher N and DOC contents, lost 6 to 8 times more C as CO_2 compared to wheat residue. Under rye and wheat amendment, absolute C losses as CO_2 (calculated per g dry soil) increased linearly with C concentration while relative C losses as CO_2 (expressed as percent of initial C) increased with C concentration following a quadratic function. In low C concentration treatments (0.38–0.79% OC), DOC decreased gradually from day 3 to day 63, microbial C increased towards the end in the rye treatment or decreased only slightly with straw amendment, and microbes invested in general enzymes such as proteases and oxidative enzymes. At increasing C levels, enzyme activity shifted to degrading cellulose after 15 days and degrading microbial necromass (e.g. chitin) after 63 days. At the highest C concentrations (2.99% OC), microbial biomass peaked early in the incubation and remained high in the rye treatment and decreased only slightly in the wheat treatment. While wheat lost C as CO_2 constantly at all C concentrations, respiration dynamics in the rye treatment strongly depended on C concentration. Our results indicate that litter quality and C concentration regulate enzyme activities, DOC concentrations, and microbial respiration. The potential for non-linear relationships between soil C

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concentration and decomposition may need to be considered in soil C models and soil C sequestration management approaches.

Keywords Decomposition dynamics · Carbon concentration · Non-linear decomposition · Microbial enzymes

Introduction

Carbon (C) mineralization following microbial decomposition of soil organic matter (SOM) is the key process that transports carbon from the soil into the atmosphere. As a largely microbial process, SOM decomposition is regulated by external factors that control microbial activity and function (Prescott 2010). Besides abiotic factors, which include temperature, moisture (Conant et al. 2011; Moyano et al. 2013), nutrient and substrate availability (Fernández-Martínez et al. 2014; Manzoni et al. 2010), and substrate quality and accessibility (Hättenschwiler and Jørgensen 2010; Dungait et al. 2012), these controls include biotic interactions such as competition and predation (Bradford et al. 2014; Kaiser et al. 2014). Another widely considered but poorly understood control over decomposition is C concentration (Don et al. 2013). The prevailing assumption is that C lost as CO_2 from soil through microbial decomposition processes is a constant proportion of C inputs or substrate concentrations (C content). Such 'first-order' decomposition kinetics are typically used in well-established biogeochemical microbial-implicit models (Coleman and Jenkinson 2008; Parton et al. 1998; Schimel et al. 2001; Sierra et al. 2012).

Soil organic matter models based on first-order linear relationships of C content and soil respiration assume that the substrate is limiting mineralization and microbes have a high capacity to mineralize the substrate that is present (Schimel and Weintraub 2003). However, recent ecosystem models challenge these assumptions (Wieder et al. 2014, 2015; Abramoff et al. 2017), and conceptual studies highlight the role of resource diffusion and spatial separation in decomposition (Buchkowski et al. 2017; Allison 2005), which are partially dependent on substrate concentration. Few experimental studies have directly addressed whether soil C losses do indeed scale with SOM content. In one such study, decomposition rates and soil carbon loss decreased after a simple dilution of decomposing organic material with mineral soil (Don et al. 2013), suggesting that the abundance of microbes or their access to substrate might limit decomposition. The authors argued that spatial distance between microbes and substrate and thus the likelihood that a microbe will encounter substrate leads to the controlling effect of C concentration on decomposition. Beyond this study, little is known about the role of C concentration in decomposition and its influence on temporal decomposition dynamics and microbial decomposition strategies. Further, the interaction of C concentration and substrate quality is poorly understood.

If C concentration influences decomposition dynamics, then this would impact soil C management in agroecosystems, soil C responses to shifts in plant productivity with climate change, and C processes across depth gradients. For example, management practices that increase soil C stocks could undermine efforts to increase soil C by easing microbial limitation and stimulating CO_2 production. In fertilized agricultural systems, interactions between N availability, productivity and ultimately microbial C resource availability could further enhance this effect. Shifts in microbial nutrient limitation caused by changes in C concentration could help explain observed increased losses of C after N addition in some agricultural soils (Finn et al. 2015; c.f. Grandy et al. 2013). By developing a mechanistic understanding of how C concentration controls decomposition, we could also contribute to refining ecosystem models to better predict changes in soil C stocks.

To evaluate the role of C concentration and substrate quality on decomposition dynamics and related microbial processes, we conducted a laboratory incubation experiment with artificial soil systems. We amended a sand-soil mixture (9:1) with finely ground rye (*Secale cereale*, C/N ratio of 23) or wheat straw (*Triticum* spp., C/N ratio of 109), resulting in seven levels of C concentration for each, ranging from 0.38% total C to 2.99% total C. To determine C dynamics, we measured soil respiration over the course of the incubation and total C, total N, dissolved organic C (DOC), and microbial biomass C after 3, 15 and 63 days of incubation at constant temperature. We also measured microbial enzyme activities to determine microbial decomposition strategies. We

hypothesized that (1) higher C concentration would increase absolute (calculated per g dry soil) and relative (expressed as percent of initial C) C loss as CO_2. We further expected that (2) different substrate qualities (i.e., C/N ratio) would not influence the relationship between C concentration and C loss dynamics.

Materials and methods

Experimental setup

Microcosms were established in Mason jars and contained a 100 g mixture of sand, field soil and plant residue. Sand and field soil were mixed at one part soil to nine parts sand to achieve a starting C concentration of 0.2%. We added 0.25, 0.5, 1, 1.5, 2, 3.5, and 5 g of plant residue to the soil-sand mixture to a final weight of 100 g. We oven-dried commercially available play sand at 60 °C and sieved it to 2 mm before mixing in soil and plant residues. Soil was collected at the University of New Hampshire experimental field station Kingman Research Farm (43°11′N 70°56′W) in May 2015. Soil type at this site is a Hollis-Charlton fine sandy loam (Smith et al. 2014). To maximize accessibility, rye (*Secale cereale*) and wheat straw (*Triticum* spp.) residues were dried and ground to a powder before addition to the microcosms. Rye averaged 55.1% C and 2.4% N (C:N ratio of 23) and wheat averaged 54.6% C and 0.5% N (C:N ratio of 109) (Table 1).

The water content of the microcosms was initially adjusted to 60% of the water holding capacity (WHC) and after 2 weeks of incubation decreased to 50% WHC of the original mixture. Microcosms were incubated for a total of 63 days at constant temperature of 25 °C and constant moisture. Fifteen replicates were established for each sample. After 3, 16, and 63 days, five replicates were harvested for analysis.

Water content, water holding capacity, pH

Water content was determined gravimetrically in soil samples that were dried at 60 °C for 24 h. Water holding capacity was measured by determining the water content after saturation of soil-sand-plant residue mixtures and allowing excess water to leach gravimetrically for 2 days while preventing evaporation. The pH was determined in a 1:5 soil to water mixture using a Mettler Toledo Seveneasy pH Meter 20 (Mettler Toledo, Columbus, Ohio, USA).

Soil C and N pools

Samples for total C and N analysis were dried at 60 °C for 24 h and finely ground in a ball mill, packed in tin capsules, and measured on an elemental analyzer (Costech Instruments ECS 4010, Costech Analytical Technologies, Valencia, California, USA). Dissolved organic carbon was measured in 1 M KCl extracts using a TOC-L CPH/CPN analyzer (Shimadzu, Kyoto, Japan). Microbial biomass C was determined using chloroform fumigation extraction (Brookes et al. 1985). Samples were fumigated in a desiccator under chloroform atmosphere for 24 h in the dark and then extracted with 1 M KCl. Fumigated samples were measured on the TOC-L CPH/CPN analyzer and microbial C was calculated as the difference of fumigated samples and KCl extracts of fresh soil samples. Microbial C is presented without the use of a correction factor for extraction efficiency. Microbial C was measured after 3, 15 and 63 days of incubation. The microbial C of the added soil was 18.50 ± 3.04 μg g^{-1} DM; microbial C associated with the used plant residues could not be detected (Table 1).

Respiration

Respiration rates were measured daily for the first week and subsequently twice per week. For respiration measurements, jars were sealed and a 5 mL sample of headspace was taken immediately and again after 30 min to 2 h depending on the duration of the experiment and the C content of the samples. Gas samples were immediately injected into an Infrared gas analyzer (Li-COR LI 820, LI-COR, Lincoln, Nebraska, USA) and rates of CO_2 production were calculated from the increase of CO_2 in the headspace of the jar over time.

C loss as CO_2 over time was calculated as cumulative respiration using the respiration rates taken at a total of 19 time points in case of the wheat straw treatment. Since respiration rates in the rye treatment, especially in the first week, were high and exceeded the standard curve, respiration rates were not used to calculate C loss. Instead, the differences

Table 1 Basic soil organic matter data for inoculation soil, plant residues, and incubated mixtures at the beginning of the incubation

	Residue amount (g per 100 g)	Soil C (% of total C)	Organic C (mg g^{-1} DM)	Total N (mg g^{-1} DM)	C/N	DOC (mg g^{-1} DM)	Microbial C (μg g^{-1} DM)
Soil	–		24.55 ± 0.33	1.869 ± 0.017	13.14 ± 0.12	0.017 ± 0.001	18.50 ± 3.04
Rye residue	–		551.1 ± 34.9	23.56 ± 0.40	23.42 ± 1.48	166.1 ± 1.3	b.d.
Wheat residue	–		546.2 ± 31.0	5.040 ± 0.288	109.1 ± 5.0	29.10 ± 0.48	b.d.
Rye							
Level 1	0.25	64.0	3.826 ± 0.121	0.245 ± 0.003	15.60 ± 0.12	0.417 ± 0.003	1.845 ± 0.304
Level 2	0.5	47.0	5.198 ± 0.208	0.304 ± 0.004	17.11 ± 0.21	0.832 ± 0.005	1.840 ± 0.303
Level 3	1	30.6	7.941 ± 0.382	0.421 ± 0.006	18.88 ± 0.38	1.663 ± 0.010	1.831 ± 0.301
Level 4	1.5	22.6	10.68 ± 0.56	0.537 ± 0.008	19.88 ± 0.56	2.493 ± 0.015	1.822 ± 0.300
Level 5	2	17.9	13.43 ± 0.73	0.654 ± 0.010	20.52 ± 0.73	3.324 ± 0.020	1.812 ± 0.298
Level 6	3.5	10.9	21.66 ± 1.25	1.005 ± 0.016	21.55 ± 1.26	5.815 ± 0.036	1.785 ± 0.294
Level 7	5	7.8	29.89 ± 1.77	1.356 ± 0.022	22.05 ± 1.78	8.307 ± 0.051	1.757 ± 0.289
Wheat							
Level 1	0.25	64.2	3.814 ± 0.111	0.199 ± 0.002	19.16 ± 0.11	0.074 ± 0.001	1.845 ± 0.304
Level 2	0.5	47.2	5.173 ± 0.188	0.211 ± 0.003	24.50 ± 0.19	0.147 ± 0.002	1.840 ± 0.303
Level 3	1	30.8	7.892 ± 0.343	0.235 ± 0.005	33.52 ± 0.34	0.293 ± 0.004	1.831 ± 0.301
Level 4	1.5	22.8	10.61 ± 0.50	0.260 ± 0.006	40.86 ± 0.50	0.438 ± 0.006	1.822 ± 0.300
Level 5	2	18.0	13.33 ± 0.65	0.284 ± 0.007	46.94 ± 0.65	0.584 ± 0.007	1.812 ± 0.298
Level 6	3.5	11.0	21.49 ± 1.12	0.357 ± 0.012	60.22 ± 1.12	1.020 ± 0.013	1.785 ± 0.294
Level 7	5	7.9	29.64 ± 1.58	0.430 ± 0.016	69.01 ± 1.58	1.457 ± 0.019	1.757 ± 0.289

Initial C concentration values for microcosms are calculated from the values for the inoculation soil and the plant residues. Inoculation soil was mixed with sand 1:9 and combined with plant residue to total 100 g of total material per microcosm. We established five replicates for each level and residue. DOC is dissolved organic C. Values are average ± standard error

between the calculated initial C content and the measured C contents at day 3, day 15, and day 63 were used to estimate the total loss of C from the system and the decrease of C between the time points. Absolute C loss as CO_2 was calculated per g dry soil, while relative C loss as CO_2 was calculated as percentage of initial C in the microcosms.

Enzyme activities

Potential extracellular enzyme activities were measured, with adaptations, as described in Schnecker et al. (2015). Soils were suspended and homogenized in 100 mM sodium acetate buffer at pH 5.5 using a commercially available blender (Magic Bullet, Alchemy Worldwide, Sherman Oaks, California, USA). The soil slurry was transferred into black microtiter plates and amended with MUF (4-methylumbelliferyl) labeled substrates: β-D-glucopyranoside for β-glucosidase (BG), β-D-cellobioside for cellobiohydrolase (CBH) or *N*-acetyl-β-D-glucosaminide for *N*-acetyl-glucosaminidase (NAG). L-alanine-7-amido-4-methyl coumarin was used as substrate for alanine-amino-peptidase (AAP). Activity was measured fluorometrically (excitation 365 nm and emission 450 nm; Biotek Synergy HT, Biotek Instruments, Winooski, Vermont, USA). Phenoloxidase (POX) and peroxidase (PEX) activities were measured using L-3,4-dihydroxyphenylalanine (DOPA) as substrate and addition of H_2O_2 for determination of PEX, in a photometric assay. POX activities were than calculated as the increase in color during the incubation time of 20 h. PEX activities were calculated as the increase in color during the incubation time from the results of the wells that received H_2O_2 minus the results of the wells without H_2O_2 addition.

Statistical analyses

To evaluate differences between sampling days for each individual level of C concentration and residue type for DOC and microbial biomass C, we used one-way ANOVA and Tukey HSD as post hoc test. Before analysis, data were log-transformed or rank-normalized to meet the assumptions for ANOVA. We calculated enzyme patterns for day 15 and 63 of the incubation, as described in Schnecker et al. (2015) to identify differences between residue type, day of sampling and C content level. To account for inherent differences in the methods to measure enzyme activities and to focus on the pattern of relative activities rather than the magnitude of enzyme activities, individual enzyme activities per gram dry soil were log transformed and standardized by calculating the relative activity of each enzyme as a proportion of the sum of all enzyme activities. We assessed the effects of residue type, day of sampling, and C concentration on enzyme activities with Permutational Multivariate Analysis of Variance (PerMANOVA; Anderson 2001). Prior to analysis, we calculated a Euclidean distance matrix from the standardized enzyme activity data. Factors in the PerMANOVA model were [distance matrix $\sim$ C concentration level $\times$ day of sampling $\times$ residue type]. To visualize the enzyme activity data, we used Nonmetric Multidimensional Scaling (NMDS). Twenty runs of the ordination (at random starting configurations and with a maximum of 200 iterations per run) were performed with an instability criterion of 0.0001. The runs were compared with 20 randomized runs to assess the significance of the reduction in stress from four dimensions to one dimension. PerMANOVA and NMDS were performed using ADONIS and vegdist and metaMDS functions, respectively, in the vegan package in R (Oksanen et al. 2016). Differences and correlations were assumed to be significant at $p < 0.05$. Statistics were performed in R 3.3.2 (R Core team 2015) and SigmaPlot 12.5 (Systat Software Inc., San Jose, California).

Results

C loss dynamics

All treatments lost significant amounts of C as CO_2 (CO_2–C) after incubation for 63 days. For both plant residue types, absolute CO_2–C losses increased linearly with initial C content (Fig. 1b). Rye-amended samples had 6–8 times greater absolute CO_2–C losses than wheat-amended samples over the 63-day incubation period. In contrast to the linear relationship between initial C content and absolute CO_2 loss, we found a quadratic relationship between initial C content and CO_2 loss expressed as a percentage of initial C in the microcosms; this was true for both residue treatments (Fig. 1a). These relative losses over the 63-day incubation period ranged from 41.5% of initial C content (0.38% OC) to 78.5% (2.96% OC) in the rye treatments and from 5.8% (0.38% OC) to 10.6% (2.99% OC) of the wheat straw treatment (Fig. 2). In the rye treatment, amendment level affected the temporal dynamics of CO_2–C loss (Fig. 2a). At low initial OC concentrations (0.38–0.52% OC), most C was lost in the first 3 days and in the period from day 15 to day 63, while comparatively little C was lost between day 3 and 15. At intermediate initial OC concentrations (0.79–2.15% OC), a large proportion of CO_2–C was lost between day 3 and 15. At the highest initial OC concentration (2.96% OC), most C was lost between day 15 and 63 while only 17% and 20% were lost in the first three days and between day 3 and day 15, respectively (Fig. 2b). Carbon loss dynamics were more evenly distributed over time in the wheat straw treatment and did not differ among levels (Fig. 2b).

Respiration rates

Respiration rates generally decreased over the course of the incubation experiment. Overall, rye-amended microcosms had higher respiration rates than those with wheat straw additions (Fig. S1). The relationship between respiration rates and C content of the microcosms changed over time and by residue treatment. In the wheat straw treatment respiration rates and C content were linearly related only at day 3 and day 15 (Fig. S1 d, e), while at day 63 respiration rates were low across concentrations (Fig. S1f). In the rye treatment, respiration rates at day 3 were highest at level 3 and decreased with higher levels of C content (Fig. S1a). Respiration at initial OC concentrations of 2.15% at day 15, and 2.96% at day 15 and day 63, were relatively high and did not follow the near-linear relationship with C content found at lower concentrations (Figs. S1c, S2b).

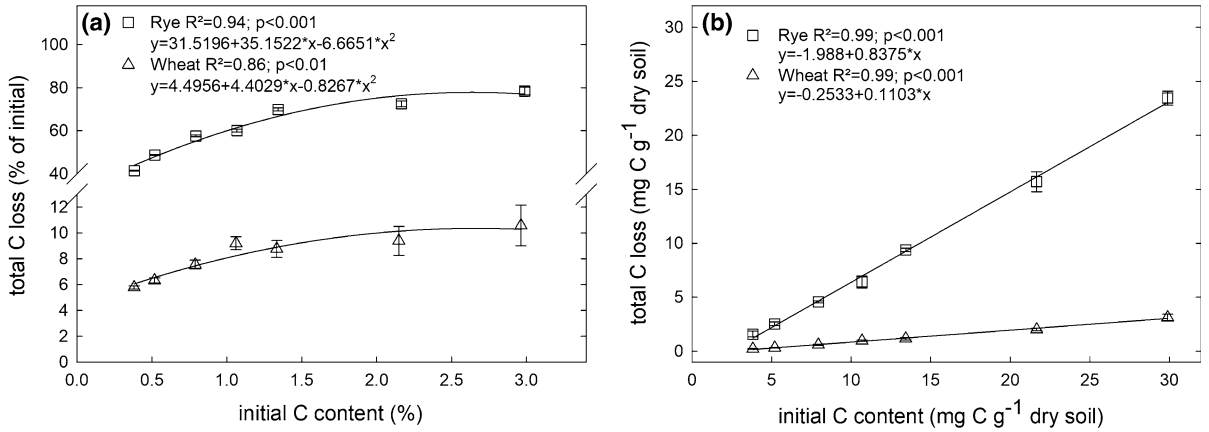

Fig. 1 Total loss of C as CO_2 from the microcosms after 63 days of incubation relative to the initial C content of the microcosms. Left panel: losses expressed as % of the initial C. For both rye and wheat straw additions, C losses follow a quadratic relationship to initial C content. Right panel: absolute losses, which are linearly related to initial C content. Values for microcosms amended with rye residue are squares and values for wheat straw amendments are triangles

DOC and microbial biomass C dynamics

Pools of extractable organic carbon and microbial biomass carbon (expressed relative to total soil C content) changed over the course of the experiment differently between residues and among initial C concentrations in the microcosms (Fig. 3). In treatments with low initial rye concentrations (0.38–0.79% OC), relative DOC concentrations gradually decreased from day 3 to day 15, and from day 15 to 63 (Fig. 3a–c). DOC concentrations were four times lower at day 3 than calculated initial DOC levels in these treatments; meanwhile, the relative pool of microbial C either did not change or slightly decreased from day 3 to day 15, and significantly increased at the end of the experiment. In rye at moderate-to-high initial OC concentrations (1.06–2.15%, Fig. 3d–f), DOC decreased significantly and steeply from day 3 to day 15 and remained constant thereafter. Microbial C at moderate initial OC concentrations (1.06–1.33%, Fig. 3d, e) also steeply decreased from day 3 to 15, although this decrease was only statistically significant at 1.06% OC. At these moderate initial OC concentrations, DOC contents did not change significantly from day 15 to 63 (Fig. 3d, e). Microbial C at high initial OC concentrations (2.15%, Fig. 3f) decreased gradually but not significantly from day 3 to day 15 and to day 63. At the highest initial OC concentrations of 2.96%, DOC was constant from day 3 to day 63 (Fig. 3g); at day 3 DOC was around 45% lower than the calculated initial DOC. In the highest OC treatment, microbial C did not change from day 3 to day 15, and at days 15 and 63 DOC was significantly greater than in other OC treatment levels on the same days.

In wheat straw treatments, DOC levels were around five times lower than in rye treatments (Fig. 3). As in the low rye treatments, changes in DOC were slight and gradual in wheat straw incubations with low initial OC concentrations (Fig. 3h, i). At 0.38% OC, DOC content increased slightly from day 3 to day 63, while at 0.52% DOC decreased from day 3 to 15 and remained stable afterwards. In contrast to the low rye treatments, microbial C peaked at 15 days in wheat straw with low initial OC concentrations of 0.38–0.52%. In treatments with wheat straw at moderate initial OC concentrations of 0.79–2.17%, DOC decreased steeply from day 3 to day 15 then either increased slightly at OC concentrations of 0.79% or did not change until day 63 (Fig. 3j–m). Microbial C did not change over time at moderate initial OC concentrations of 1.07%. At high initial OC concentrations of 1.34–2.99% microbial C decreased steeply from day 3 to day 15 (Fig. 3l–n), although this was not significant at 1.34% OC, followed by no significant change from day 15 to day 63. DOC at 2.99% OC, the highest concentration, decreased more gradually over the course of the incubation, such that DOC levels remained rather high from day 3 to 63.

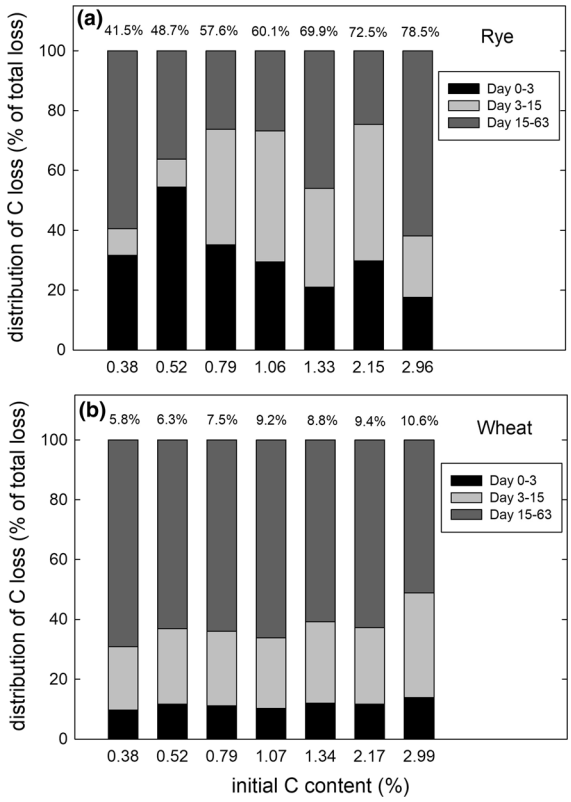

Fig. 2 Distribution of gaseous C loss. Shading indicates in which period of time the given proportion of the total C loss was released from the soil-sand-residue mixture: black for the first 3 days of the incubation, light grey day 3–15, and dark grey day 15–63. The numbers above the bars indicate the amount of initial C lost from the microcosms of the respective level during the 63-day incubation. **a** Microcosms with rye addition; **b** microcosms with wheat straw addition

Microbial enzyme activities

Microbial enzyme patterns, expressed as a distance matrix calculated from log-transformed and standardized potential enzyme activities, represented the microbial foraging strategy. These patterns differed significantly between residue types, day of incubation, and among C content levels (Table 2). Enzyme patterns in rye treatments followed patterns in C content. Low initial OC concentrations were associated with AAP, POX and PEX activities, whereas treatments with high OC concentrations were associated with BG and CBH activities (Fig. 4a). Enzyme patterns at high and low initial C concentrations differed from inoculation soil. After 63 days, low C levels were still associated with AAP, POX and PEX but higher C levels became more associated with NAG than BG and CBH (Fig. 4a). Wheat straw treatments generally had similar enzyme patterns to rye treatments, although not as pronounced. Especially at lower C levels, samples from day 15 differed from the day 63 samples, which shifted towards PEX and NAG activities (Fig. 4b).

Discussion

The role of soil carbon concentration in determining microbial activity and C fluxes is potentially important for soil C dynamics, ecosystem modelling and managing soil C sequestration. In our 63-day laboratory incubation, we found that the magnitude of CO_2 loss from the system was associated with substrate quality, especially the initial N and DOC content of the plant residues. Beyond substrate quality, C concentration itself impacted both the absolute and relative losses of C as CO_2 from the soil system (Fig. 1a), and decomposition dynamics and microbial decomposition strategies both depended on C concentration. The effect of C concentration on decomposition dynamics was most apparent in the rye-amended mesocosms, so we begin by describing decomposition dynamics in this substrate treatment.

Decomposition at low C concentrations

Most of the C lost from microcosms amended with rye at low C concentrations (OC 0.38–0.52%) was respired in the first 3 days and the last 48 days of the incubation (days 15 to 63; Fig. 2a). These treatments, as well as the rye treatment with 0.79% C, lost most of their DOC as CO_2 soon after the incubation began, with DOC further decreasing gradually between day 3 and 63 (Fig. 3a–c). However, microbial biomass did not increase until the end of the incubation (between day 15 and 63), and even then only slightly (Fig. 3a–c). This late increase in microbial biomass might represent slower-growing filamentous microbes such as fungi (Boddy et al. 2009) that could grow into previously untapped pockets of plant residue over the course of several weeks or months (Fig. 5).

In addition to these decomposition dynamics, oxidative enzymes (POX and PEX) and proteases (AAP) contributed more to total enzyme activity at the lowest rye C concentration (0.38%) after 15 days compared to the inoculation soil (Fig. 4a). These are

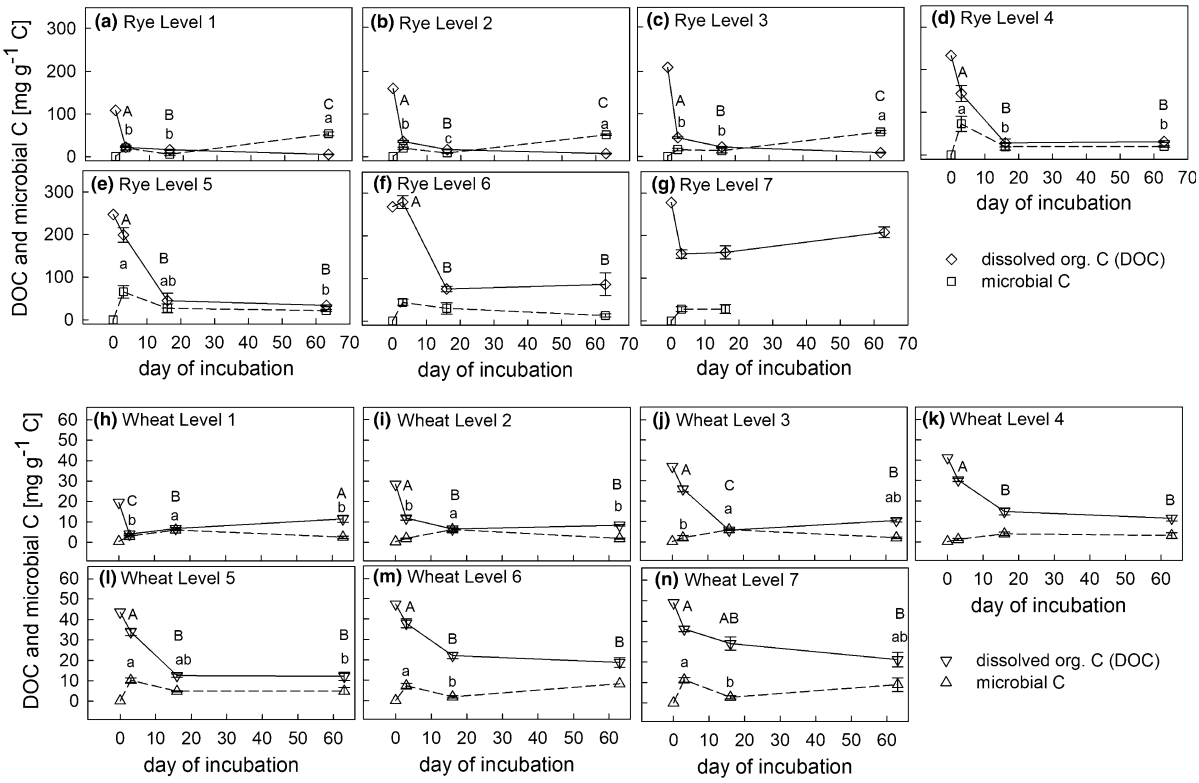

Fig. 3 Temporal dynamics of dissolved organic C (DOC) and microbial C at different C concentration levels. Top block: rye residue treatments; diamonds represent DOC and squares represent microbial C. Bottom block: wheat straw residue treatments; down-pointing triangles represent DOC and up-pointing triangles represent microbial C. All values are calculated per g C. Capital letters indicate significant differences between DOC contents at day 3, 15, and 63. Lower case letters indicate differences between microbial C at the different time points. Because the points at day 0 were not measured but rather calculated from the inoculation soil and plant residues values, day 0 was not considered in the statistical analyses

Table 2 Results of PerMANOVA on enzyme activity data as shown in Fig. 4

	Rye + wheat	Rye	Wheat
Residue type	0.11	–	–
Harvest (incubation day)	0.09	0.27	0.27
Level	0.29	0.49	0.32

Values are correlation coefficients (r^2). All three effects had p values < 0.001 on the enzyme patterns for each of the three treatments

generalist enzymes: proteases are widely distributed amongst soil microbes (Geisseler et al. 2010), while oxidative enzymes are less substrate-specific than hydrolytic enzymes (Baldrian 2006; Sinsabaugh 2010). For example, peroxidases produce oxygen radicals that can attack a particularly diverse array of substrates (Sinsabaugh 2010). Microbes often switch to oxidative enzymes to degrade complex organic material after labile organic matter is depleted (Rinkes et al. 2013; McDaniel et al. 2014). In deep soils more complex substrate chemistry, other enzyme stabilization mechanisms (Kramer et al. 2013), or a different microbial community composition than in the surface soil have been used to explain relatively higher oxidative enzyme activities (Schnecker et al. 2015; Stone et al. 2014). In our incubation, however, we also found a shift towards oxidative enzymes at low C concentrations, although the experiment was started with the same microbial community and the same substrate at all C concentration levels. This points to a strong effect of C concentration on enzyme patterns and thus microbial acquisition strategy. The combination of a potentially less efficient set of enzymes to degrade plant material, i.e. oxidative enzymes, and a late increase in microbial biomass, might account for the lower relative losses of C as CO_2 from microcosms with low C content (Fig. 1a).

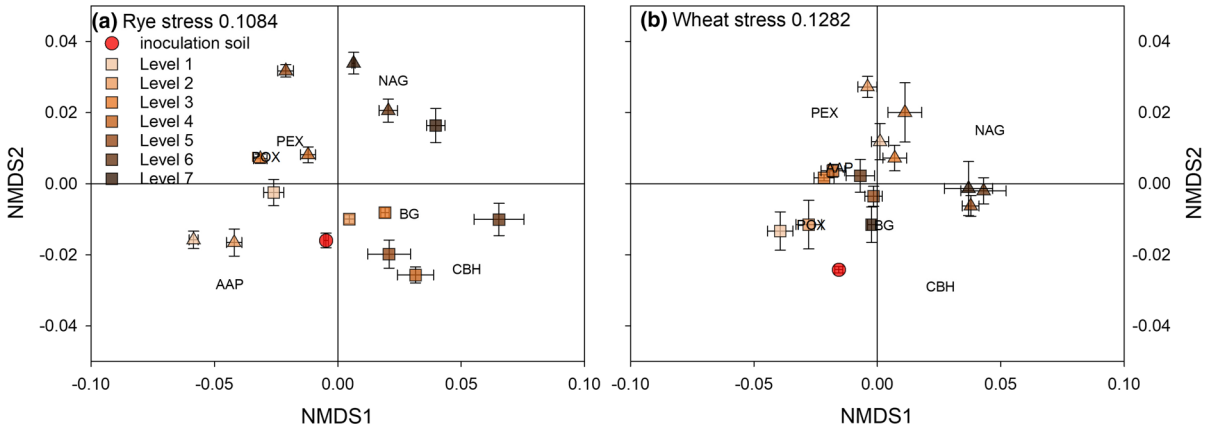

Fig. 4 NMDS plots of standardized enzyme activities. Symbols represent mean values of replicated microcosms. Color shades correspond to the 7 different C levels of the microcosms at the start of the incubation. Squares represent values from samples at day 15 of the incubation, triangles represent samples from day 63, and the red circle represents the enzyme patterns of the inoculation soil. Left panel: samples from microcosms with rye addition; right panel: samples from wheat straw amendments. *AAP* alanine-amino-peptidase, *POX* phenoloxidase, *PEX* peroxidase, *NAG N*-acetyl glucosaminidase, *BG* beta-glucosidase, *CBH* cellobiohydrolase. (Color figure online)

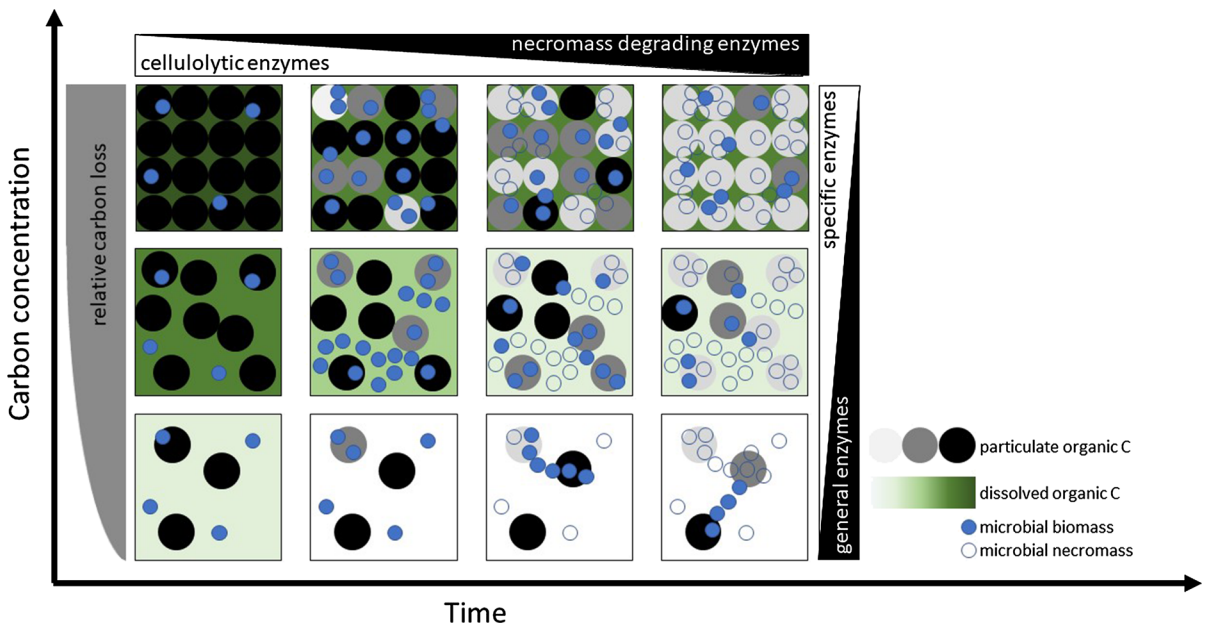

Fig. 5 Conceptual summary of the relationships between carbon dynamics over time and initial C concentration. Large grey and black cycles indicate particulate organic C (POC) in increasing concentration from light to dark. The hue of the background represents dissolved organic C (DOC) concentration with white and light green for low concentrations, and dark green for high concentrations. Small blue cycles are living microbes and white cycles symbolize microbial necromass. (Color figure online)

Decomposition at intermediate C concentrations

In contrast to microcosms with low C concentrations, microcosms with intermediate organic C concentrations (OC 0.79–2.17%) lost most of their C between days 3 and 15 (Fig. 2a). DOC steeply decreased from day 3 to day 15 and subsequently remained stable (OC 1.07–2.17%), while microbial C peaked at day 3 (Fig. 3d–f), sharply contrasting with the late-incubation increase in microbial biomass we observed at low C concentrations. This initial peak in microbial C suggests that microbial biomass was able to bloom

because DOC was still abundant at day 3 in intermediate C concentrations, in contrast to microcosms with low C concentrations which had much lower DOC concentrations by day 3. The consistently high gaseous C losses from day 3 to day 15 were likely driven by production of new enzymes, which often lags behind substrate addition (Rinkes et al. 2014). Unlike the more general set of enzymes at low C concentrations, treatments with intermediate C concentrations had a high proportion of cellulolytic enzyme activities at day 15 (Fig. 4a), suggesting that decomposition of the amended plant material in these microcosms was more specific and efficient. By day 63, DOC had been exhausted and microbial biomass decreased; at the same time, enzyme patterns shifted towards the cell-wall degrading enzyme NAG, possibly indicating that microbes were using necromass as an alternative substrate for growth.

Decomposition at high C concentrations

In microcosms with the highest C concentration (OC 2.99%) both the even distribution of gaseous C losses (Fig. 2a) and the consistently high DOC concentrations (Fig. 3g), could indicate that while DOC was consumed, the DOC pool was constantly replenished by the degradation of the amended plant material. This is consistent with the high proportion of cellulolytic enzyme activities at day 15 (Fig. 4a). A constant supply of DOC might also have promoted consistent microbial biomass production and turnover at OC concentrations of 2.17% (Fig. 3f). Similar dynamics in DOC and microbial C pools were observed at 2.99% OC, although the microbial C values for day 63 are missing for this treatment (Fig. 3g). A constant decomposition of amended plant material and potentially a high microbial biomass turnover could help to explain the relatively high activities of NAG after only 15 days (Fig. 4a), which suggest that necromass became a substrate for microbial uptake.

The role of substrate quality in the C concentration effect

Wheat straw residue treatments were broadly similar to rye residue treatments, with some exceptions. Relative C losses as CO_2 were 6–8 times higher in the rye treatment (Fig. 1). DOC and microbial biomass dynamics over time varied with C content in both substrate treatments (Fig. 3), but the absolute amounts of DOC and microbial biomass were substantially lower in the wheat straw compared to the rye treatment.

The largest differences between the residues were in the temporal patterns of C loss as CO_2 (Fig. 2). In contrast to rye, losses from wheat straw were more evenly distributed throughout the incubation and across C concentrations (Fig. 2b). Respiration rates in the wheat straw treatments were linearly related to C content at day 3 and day 15 (Fig. S1) whereas respiration rates in the rye microcosms were never linearly related to C content. Enzyme patterns in the wheat straw treatment varied with C concentration and day of the incubation (Table 2), and changed gradually—although not as distinctly—as in rye (Fig. 3). The low N content and a lower proportion of DOC in wheat straw could explain why it behaved differently than the rye treatment (Table 1). Reduced nutrient availability might have restricted microbial growth (Manzoni et al. 2010), substrate use, and total gaseous C loss (Manzoni et al. 2012; Sinsabaugh et al. 2013), which could further explain the different patterns in C loss as CO_2, DOC, microbial biomass, and enzyme production.

Concepts behind the effect of C concentration on decomposition

Substrate type, microbial community properties, or substrate-microbe interactions could drive the effect of C concentration on decomposition. In this experiment we mixed soil with sand and plant material in increasing concentrations with the result that native soil organic C contributed more to total soil C at low initial C concentrations than high concentrations. Because some native SOC is associated with minerals, a higher contribution of native soil C to total SOC could explain the lower relative CO_2–C losses in the low-concentration rye treatments. However, we also found the same effect with straw, which had a C:N ratio of 109 compared to the soil C:N ratio of 13. In this case, much of the native SOC would be higher quality than the litter and should be preferentially used in the short-term, even if part of the soil C is bound to minerals. Still the general patterns that C concentration effects decomposition could be found for rye and wheat treatments.

Alternately, microbial community adaptation, such as changes in community composition, physiology,

and/or decomposition strategies, could explain the effects of C concentration on decomposition. For example, if microbial C use efficiency varies with substrate availability across the C gradient, non-linear C dynamics could emerge (Eiler et al. 2003; Kallenbach et al. 2015). As we increased substrate concentrations we could also have stimulated different members of the microbial community (Reischke et al. 2014) which could, through variations in physiology, feed back to decomposition dynamics (Waring et al. 2013).

The observed non-linear control of substrate concentration on C loss dynamics and microbial decomposition strategies could also be related to the spatial distribution of microbes and their substrate and the average distance between them (Don et al. 2013; Falconer et al. 2015; Ruamps et al. 2013; Schimel and Schaeffer 2012; Vogel et al. 2015). Microbes and particulate organic carbon (POC) occupy a defined soil space. In soil with an initially even distribution of microbes, higher amounts of similarly distributed particulate organic matter should increase the probability that microbes encounter their substrates. As substrate density increases it could shift the limitation on decomposition away from one of diffusion—and thus substrate—limitation (Vetter et al. 1998), and toward a microbial or enzymatic limitation (Schimel and Weintraub 2003) at high substrate concentrations and microbe density (Buchkowski et al. 2017). We conceptually illustrate how spatial dynamics could influence microbe-substrate interactions and create the patterns of decomposition dynamics and strategies we observed in our incubation experiment (Fig. 5).

At low C concentrations, few microbes have access to POC while all have access to low concentrations of DOC. Most DOC is exhausted as decomposition begins and microbes increase their biomass to reach untapped POC. At these low C concentrations minimal C is lost from the system, in part because microbes cannot reach all the POC until later stages of decomposition, but also because they predominantly produce oxidative rather than hydrolytic enzymes. At intermediate C concentrations, early decomposition is characterized by a microbial bloom. This enables microbes to colonize most of the POC, which leads to more production of DOC and large losses of C from the system. Microbial biomass decreases once DOC is exhausted, but C losses during the mid-stages of decomposition will be high because most of the POC is colonized. With time, microbial necromass gains importance as substrate for microbial growth. At high C concentrations, microbes reach most POC early on. Microbial biomass growth is fueled by DOC and POC. Since microbes have already degraded most of the POC, DOC decreases only in the very early stages of decomposition and remains stable afterwards. The fast colonization of POC also leads to a faster exhaustion and an early increase of necromass-degrading enzymes.

Despite the lack of data on how the in situ spatial distribution of substrates and microbes affects microbial community composition, the theoretical concept we outline above could explain the relationships we observed between C concentration, decomposition dynamics, and microbial decomposition strategies. Further, this framework can help structure direct testing of spatial controls on decomposition dynamics. Our framework combines three key aspects of decomposition: (1) the initial probability that microbes encounter their substrate (Don et al. 2013; Buchkowski et al. 2017), (2) how varying DOC inputs affect diffusion time and microbial motility (Vetter et al. 1998; Allison 2005; Jimenez-Sanchez et al. 2015), and (3) temporal changes in substrate use with substrate availability, as reflected in enzyme patterns.

In summary, the magnitude of soil C loss as CO_2 from artificial soil systems was mainly related to N and DOC concentrations of the different plant residue amendments. However, C concentration modulated C loss dynamics, changes in DOC and microbial biomass over time, and microbial decomposition strategies such as patterns of enzyme activity. Our results further indicate that, contrary to the predominant assumption, C loss is not a fixed proportion of C content or input; this should be tested in natural soils and potentially given consideration in ecosystem models. In addition, our findings might alter how we manage soil systems for C sequestration (Minasny et al. 2017; Ryals et al. 2015; Smith 2016), considering that even small differences in initial C content lead to very different decomposition dynamics.

Many questions remain about the effect of C concentration on soil organic matter decomposition, as our study is one of only a few on this topic. Further, because we used an artificial soil system under optimal temperature and moisture conditions, the open question of how microbial community dynamics and physiology will respond to the spatial distribution of microbes and substrate in natural soil systems is so far

unanswered. Nevertheless, our results reveal that complex relationships between C inputs and losses can emerge from the interplay of space, microbial community composition dynamics, and microbial decomposition strategies under different resource concentrations.

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References

Abramoff R, Xu X, Hartman M, O'Brien S et al (2017) The Millennial model: in search of measureable pools and transformations for modeling soil carbon in the new century. Biogeochemistry 137:51. https://doi.org/10.1007/s10533-017-0409-7

Allison SD (2005) Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecol Lett 8:626–635. https://doi.org/10.1111/j.1461-0248.2005.00756.x

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Baldrian P (2006) Fungal laccases—occurrence and properties. FEMS Microbiol Rev 30:215–242. https://doi.org/10.1111/j.1574-4976.2005.00010.x

Boddy L, Hynes J, Bebber DP, Fricker MD (2009) Saprotrophic cord systems: dispersal mechanisms in space and time. Mycoscience 50:9–19. https://doi.org/10.1007/s10267-008-0450-4

Bradford MA, Wood SA, Bardgett RD et al (2014) Discontinuity in the responses of ecosystem processes and multifunctionality to altered soil community composition. Proc Natl Acad Sci USA 111:14478–14483. https://doi.org/10.1073/pnas.1413707111

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842. https://doi.org/10.1016/0038-0717(85)90144-0

Buchkowski RW, Bradford MA, Grandy AS et al (2017) Applying population and community ecology theory to advance understanding of belowground biogeochemistry. Ecol Lett 20:231–245. https://doi.org/10.1111/ele.12712

Coleman K, Jenkinson DS (2008) RothC 26.3: a model for the turnover of carbon in soil November 1999 issue (modified August 2008). Rothamsted Res 47

Conant RT, Ryan MG, Ågren GI et al (2011) Temperature and soil organic matter decomposition rates—synthesis of current knowledge and a way forward. Glob Change Biol 17:3392–3404. https://doi.org/10.1111/j.1365-2486.2011.02496.x

Don A, Rödenbeck C, Gleixner G (2013) Unexpected control of soil carbon turnover by soil carbon concentration. Environ Chem Lett 11:407–413. https://doi.org/10.1007/s10311-013-0433-3

Dungait JAJ, Hopkins DW, Gregory AS, Whitmore AP (2012) Soil organic matter turnover is governed by accessibility not recalcitrance. Glob Change Biol 18:1781–1796

Eiler A, Langenheder S, Bertisson S, Tranvik LJ (2003) Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. Appl Environ Microbiol 69:3701–3709. https://doi.org/10.1128/AEM.69.7.3701

Falconer RE, Battaia G, Schmidt S et al (2015) Microscale heterogeneity explains experimental variability and non-linearity in soil organic matter mineralisation. PLoS ONE 10:1–12. https://doi.org/10.1371/journal.pone.0123774

Fernández-Martínez M, Vicca S, Janssens IA et al (2014) Nutrient availability as the key regulator of global forest carbon balance. Nat Clim Change 4:471–476. https://doi.org/10.1038/nclimate2177

Finn D, Page K, Catton K et al (2015) Soil biology & biochemistry effect of added nitrogen on plant litter decomposition depends on initial soil carbon and nitrogen stoichiometry. Soil Biol Biochem 91:160–168. https://doi.org/10.1016/j.soilbio.2015.09.001

Geisseler D, Horwath WR, Joergensen RG, Ludwig B (2010) Pathways of nitrogen utilization by soil microorganisms—a review. Soil Biol Biochem 42:2058–2067. https://doi.org/10.1016/j.soilbio.2010.08.021

Grandy AS, Salam DS, Wickings K, McDaniel M, Culman SW, Snapp SS (2013) Soil respiration and litter decomposition responses to nitrogen fertilization rate in no-till corn systems. Agric Ecosyst Environ 179:35–40. https://doi.org/10.1016/j.agee.2013.04.020

Hättenschwiler S, Jørgensen HB (2010) Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. J Ecol 98:754–763. https://doi.org/10.1111/j.1365-2745.2010.01671.x

Jimenez-Sanchez C, Wick LY, Cantos M, Ortega-Calvo JJ (2015) Impact of dissolved organic matter on bacterial tactic motility, attachment, and transport. Environ Sci Technol 49:4498–4505. https://doi.org/10.1021/es5056484

Kaiser C, Franklin O, Dieckmann U, Richter A (2014) Microbial community dynamics alleviate stoichiometric constraints during litter decay. Ecol Lett 17:680–690. https://doi.org/10.1111/ele.12269

Kallenbach CM, Grandy AS, Frey SD, Diefendorf AF (2015) Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biol Biochem 91:279–290. https://doi.org/10.1016/j.soilbio.2015.09.005

Kramer S, Marhan S, Haslwimmer H et al (2013) Temporal variation in surface and subsoil abundance and function of

the soil microbial community in an arable soil. Soil Biol Biochem 61:76–85. https://doi.org/10.1016/j.soilbio.2013.02.006

Manzoni S, Trofymow JA, Jackson RB, Porporato A (2010) Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecol Monogr 80:89–106. https://doi.org/10.1890/09-0179.1

Manzoni S, Taylor P, Richter A et al (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol 196:79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x

McDaniel MD, Grandy AS, Tiemann LK, Weintraub MN (2014) Crop rotation complexity regulates the decomposition of high and low quality residues. Soil Biol Biochem 78:243–254. https://doi.org/10.1016/j.soilbio.2014.07.027

Minasny B, Malone BP, McBratney AB et al (2017) Soil carbon 4 per mille. Geoderma 292:59–86. https://doi.org/10.1016/j.geoderma.2017.01.002

Moyano FE, Manzoni S, Chenu C (2013) Responses of soil heterotrophic respiration to moisture availability: an exploration of processes and models. Soil Biol Biochem 59:72–85

Oksanen J, Blanchet F, Kindt R et al (2016) Vegan: community ecology package. R Packag. 2.3-3 https://cran.r-project.org/web/packa

Parton WJ, Hartman M, Ojima D, Schimel D (1998) DAYCENT and its land surface submodel: description and testing. Glob Planet Change 19:35–48. https://doi.org/10.1016/S0921-8181(98)00040-X

Prescott CE (2010) Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101:133–149. https://doi.org/10.1007/s10533-010-9439-0

R Core team (2015) R A Lang. Environ. Stat. Comput. R Found. Stat. Comput. R Core team, Vienna

Reischke S, Rousk J, Bååth E (2014) The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biol Biochem 70:88–95. https://doi.org/10.1016/J.SOILBIO.2013.12.011

Rinkes ZL, Sinsabaugh RL, Moorhead DL, Grandy AS, Weintraub MN (2013) Field and lab conditions alter microbial enzyme and biomass driving decomposition of the same leaf litter. Front Microbiol 4:1–14. https://doi.org/10.3389/fmicb.2013.00260

Rinkes ZL, DeForest JL, Grandy AS et al (2014) Interactions between leaf litter quality, particle size, and microbial community during the earliest stage of decay. Biogeochemistry 117:153–168. https://doi.org/10.1007/s10533-013-9872-y

Ruamps LS, Nunan N, Pouteau V et al (2013) Regulation of soil organic C mineralisation at the pore scale. FEMS Microbiol Ecol 86:26–35. https://doi.org/10.1111/1574-6941.12078

Ryals R, Hartman MD, Parton WJ et al (2015) Long-term climate change mitigation potential with organic matter management on grasslands. Ecol Appl 25:531–545

Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. Front Microbiol 3:1–11. https://doi.org/10.3389/fmicb.2012.00348

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563

Schimel DS, House JI, Hibbard KA et al (2001) Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. Nature 414:169–172. https://doi.org/10.1038/35102500

Schnecker J, Wild B, Takriti M et al (2015) Microbial community composition shapes enzyme patterns in topsoil and subsoil horizons along a latitudinal transect in Western Siberia. Soil Biol Biochem 83:106–115. https://doi.org/10.1016/j.soilbio.2015.01.016

Sierra CA, Müller M, Trumbore SE (2012) Models of soil organic matter decomposition: the SoilR package, version 1.0. Geosci Model Dev 5:1045–1060. https://doi.org/10.5194/gmd-5-1045-2012

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42:391–404

Sinsabaugh RL, Manzoni S, Moorhead DL, Richter A (2013) Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecol Lett 16:930–939. https://doi.org/10.1111/ele.12113

Smith P (2016) Soil carbon sequestration and biochar as negative emission technologies. Glob Change Biol 22:1315–1324. https://doi.org/10.1111/gcb.13178

Smith RG, Atwood LW, Warren ND (2014) Increased productivity of a cover crop mixture is not associated with enhanced agroecosystem services. PLoS ONE 9(5):e97351. https://doi.org/10.1371/journal.pone.0097351

Stone MM, DeForest JL, Plante AF (2014) Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. Soil Biol Biochem 75:237–247. https://doi.org/10.1016/j.soilbio.2014.04.017

Vetter YA, Deming JW, Jumars PA, Krieger-Brockett BB (1998) A predictive model of bacterial foraging by means of freely released extracellular enzymes. Microb Ecol 36:75–92

Vogel LE, Makowski D, Garnier P et al (2015) Modeling the effect of soil meso- and macropores topology on the biodegradation of a soluble carbon substrate. Adv Water Resour 83:123–136. https://doi.org/10.1016/j.advwatres.2015.05.020

Waring BG, Averill C, Hawkes CV (2013) Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. Ecol Lett 16:887–894. https://doi.org/10.1111/ele.12125

Wieder WR, Grandy AS, Kallenbach CM, Bonan GB (2014) Integrating microbial physiology and physio-chemical principles in soils with the Microbial-Mineral Carbon Stabilization (MIMICS) model. Biogeosciences 14:3899–3917. https://doi.org/10.5194/bg-11-3899-2014

Wieder WR, Grandy AS, Kallenbach CM, Taylor PG, Bonan GB (2015) Representing life in the earth system with soil microbial functional traits in the MIMICS model. Geosci Model Dev 6:1789–1808. https://doi.org/10.5194/gmd-8-1789-2015