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Mechanism of Positive, Non-Additive Litter Decomposition

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Doctor of Philosophy

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ABSTRACT

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Litter decomposition is a fundamental ecosystem process. It is responsible for nutrient cycling and influences carbon (C) sequestration, and soil physical and chemical properties. In nature, litter is usually heterogeneous and may not decompose the way homogeneous litter does. For example, heterogeneous litter decomposition is frequently non-additive. This makes the rate of nutrient cycling as well as fluxes of C into and out of soil C pools impossible to predict. The most frequently proposed mechanisms for positive, non-additive decomposition include the supply of limiting mineral nutrients, the supply of available C (priming), and the improvement of micro-environmental conditions. However, all three mechanisms are controversial in the sense that no single mechanism accounts for all cases of non-additive decomposition. In mesic ecosystems, both soil microbes and soil fauna are the major causes of decomposition. Microbes decompose litter by producing extracellular enzymes. The comminution of litter by soil animals interacts with microbial activities by increasing substrate surface area. In our study, positive, non-additive decomposition of oat straw when mixed with clover was not due to enhancing microarthropod density in oat straw but associated with significantly increased microbial activity in oat straw. We further investigated the factors that contribute to positive, non-additive decomposition by testing several common hypotheses used to explain non-additive decomposition (increased water content, and the transfer of C and/or nitrogen (N) compounds from clover to oat straw). We also tested a new hypothesis, which is that C, N and other nutrients are simultaneously supplied by clover to stimulate the decomposition of oat straw. Our study indicated that the addition of water to oat straw did not increase oat straw decomposition and adding ammonium chloride only or glucose and ammonium chloride together to oat straw had no significant effect on oat straw decomposition. Glucose addition alone (Low concentration) increased oat straw decomposition but was not sufficient to predict the effect of clover litter. Either the addition of glucose, ammonium chloride and other minerals together to oat straw, or soil was in contact with oat straw and glucose and ammonium chloride were added, oat straw decomposition was stimulated as if clover were present. These results suggest that the limiting resources are some combination of C, N and other mineral nutrients and that soil itself may be a source of limiting nutrients in litter decomposition. In nature, some combination of high quality litter and soil itself may supply resources that stimulate the decomposing organisms' activity on low quality litter and then the decomposition of low quality litter. Our research provides insight into the dynamics of heterogeneous litter decomposition and will allow us to better model nutrient cycling.

Keywords: litter mixture, positive, non-additive decomposition, microarthropod density, microbial activity, C transfer, mineral nutrient transfer

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CHAPTER 1: Review of positive non-additive litter decomposition

INTRODUCTION

Litter decomposition is a fundamental ecosystem process. It converts organic molecules containing elements such as nitrogen (N), phosphorus (P), and sulfur (S) into mineral nutrients that are necessary for plant growth (Gartner and Cardon 2004). It is also the process by which organic carbon (C) is mineralized into CO_2 (Berg and McClaugherty 2014). During the process, however, some highly recalcitrant soil organic matter (SOM) is formed, which sequesters substantial amounts of C in the soil (Cotrufo et al. 2010). SOM concentration is an important indicator of soil quality (Reeves 1997) because it impacts soil fertility (Hargitai 1993), water-holding capacity (Carter 2002, Díaz-Zorita et al. 1999), aeration, and aggregate stability (Piccolo 1996).

Currently, I have a good understanding of factors influencing decomposition of homogeneous litter, including climate (Couteaux et al. 1995, Aerts 1997), litter physical and chemical properties (Hättenschwiler and Jørgensen 2010, Makkonen et al. 2013), and soil faunal and microbial communities (Seastedt 1984, Beare et al. 1992, Schneider et al. 2012). However, in natural ecosystems litter is almost always found to exist in heterogeneous mixtures (Blair et al. 1990). Unfortunately, it is difficult to predict the decomposition rate of mixed litter based on the rates of homogeneous litter decomposition because mixed litter decomposition can be non-additive (Figure 1.1, Gartner and Cardon 2004). Indeed, Gartner and Cardon (2004) indicated that 67% of 30 publications examining decomposition of mixed litter demonstrated non-additive decomposition. Thus, understanding why non-additive decomposition occurs is critical to a complete understanding of the controls of nutrient cycling and C flow in ecosystems.

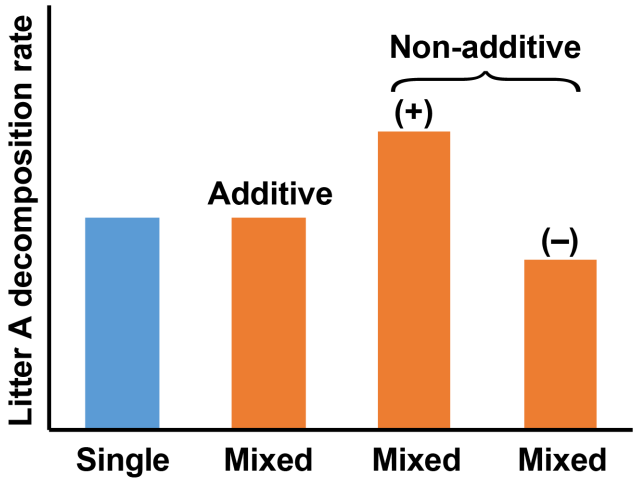

Figure 1.1. Schematization of the non-additive decomposition of mixed litter. Single = litter A alone; Mixed = litter A mixed with another type of litter.

Negative non-additive decomposition may occur because of the transfer of secondary metabolites that inhibit decomposer activity (Hättenschwiler et al. 2005). It is not our intention to discuss mechanisms by which negative, non-additive decomposition occurs. Here I discuss the most frequent mechanisms proposed for positive, non-additive decomposition. Finally, I present a synthetic conceptual model to account for positive, non-additive decomposition.

MECHANISMS OF POSITIVE NON-ADDITIVE DECOMPOSITION

In the past three decades, researchers have proposed several mechanisms to explain positive non-additive decomposition. However, none of these mechanisms appear to be operative under all circumstances. Here I review the most frequently proposed mechanisms for positive, non-additive decomposition.

Nutrient transfer

Different litters possess different chemical and physical properties. When they decompose in mixtures, decomposer fungi will often produce networks of hyphae that connect fragments of the different litters. These networks can transfer limiting nutrients from nutrient-rich litter to nutrient-poor litter (Taylor et al. 1989, Fyles and Fyles 1993, Wardle et al. 1997). Consequently, the nutritional status of nutrient-poor litter is improved and its decomposition is accelerated.

Litter is low in N when it is primarily composed of cellulose, hemicellulose, and lignin. Thus, N is frequently the nutrient most commonly limiting microbial activity during litter decomposition. This has been supported by N fertilization experiments in which adding N increased litter decomposition rate (Carreiro et al. 2000, Vestgarden 2001, Ågren et al. 2001, Vivanco and Austin 2011). Positive, non-additive decomposition of mixed litters has been shown when the two litter types differ markedly in initial N concentration and such cases have been ascribed to passive N translocation by diffusion and to active N translocation by fungal hyphae (McTiernan et al. 1997, Salamanca et al. 1998, Liu et al. 2007).

However, the relationship between litter N concentration and non-additive decomposition is still controversial. Lummer et al. (2012) used N isotopes to trace N movement between litters and found that N-poor litter may act as a N source in litter mixtures. Thus, N-rich litter sometimes has no impact on the decomposition rate of N-poor litter (Smith and Bradford 2003). In some cases this may be because N concentration does not necessarily reflect actual N availability in the litter, in other cases it possible because N is not limiting.

Some studies found that P may be the nutrient that most limits microbial litter decomposition (Montané et al. 2010, Lodge et al. 2014). For example, when Montané et al.

(2013) mixed P-rich grass litter with P-poor legume litter, they found increased P concentration and increased decomposition in the legume litter, suggesting that P transfer may have been the reason for increased decomposition of the legume litter. Obviously, insufficient P will not always limit litter decomposition. In an experiment with 15 combinations of four litter species, Ball et al. (2009) found that P dynamic of P-poor litter was often but not always affected by mixing with other litters. In other studies, the transfer of other nutrients, such as potassium, magnesium, manganese, and calcium, was associated with increased litter decomposition (Ghasemi-Aghbash et al. 2016).

Priming

Priming is the increase in organic matter decomposition resulting in C and N release from the soil due to the short term stimulation of decomposer organisms (Kuzyakov et al. 2000). This stimulation may come from the addition of labile N (Jenkinson et al. 1985), in which case priming is no different from the nutrient transfer mechanism.

Stimulation of decomposer organisms may also occur as a consequence of the addition of labile sources of C (Kuzyakov and Bol 2006, Dijkstra et al. 2006). Because approximately half the mass of plant litter is C, many have not considered C as a potentially limiting factor in the process of decomposition. However, the quality of C varies tremendously among litter types (Vogel 2008). C compounds in litter range from easily accessible compounds such as sugars and amino acids, to highly recalcitrant compounds such as condensed tannins and lignin (Hättenschwiler and Jørgensen 2010). Litter high in the latter may not meet the demand for C by decomposer organisms during decomposition. In a field study, for example, Hättenschwiler and Jørgensen (2010) found a good correlation between remaining litter mass and initial C quality,

indicating that C availability may have been the limiting factor in decomposition. For example, when *Paxillus involutus*, an ectomycorrhizal fungus, decomposes plant litter, it may be limited by available C. When glucose was supplied to the fungus, Rineau et al. (2013) showed a C priming effect on plant litter decomposition. Thus, the addition of labile C may stimulate the activity of soil organisms, eventually leading to an increase in resident organic matter decomposition (Kuzyakov and Bol 2006, Dijkstra et al. 2006). On the other hand, the addition of large quantities of labile C may cause at least a temporary reduction in the decomposition of resident organic matter as microbes initially metabolize only the labile C (Kuzyakov and Bol 2006, Chigineva et al. 2009), a process I refer to as C substitution, which can lead to negative priming. However, trace amounts of labile C and N ($\mu g\ g^{-1}$ quantities) accelerated the decomposition of cellulose (De Nobili et al. 2001). The impact of labile C concentration on priming is shown in Figure 1.2.

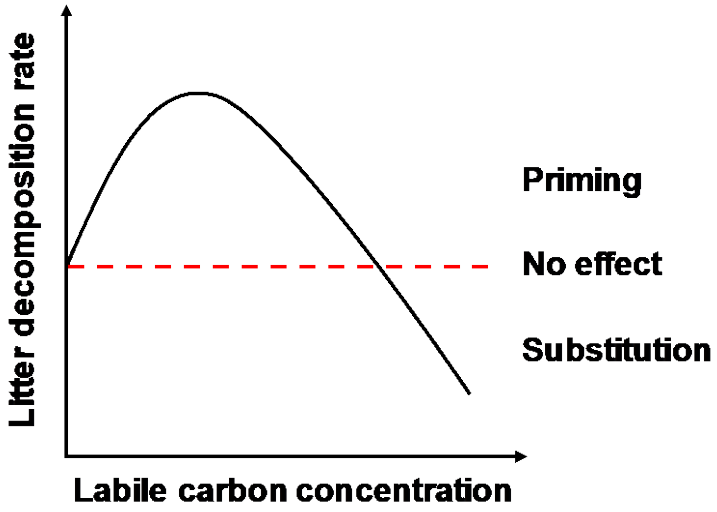

Figure 1.2. The effect of labile C on litter decomposition when decomposers are limited by available C.

Improved micro-environmental conditions

One type of litter may also increase the rate of decomposition of another type by increasing moisture availability or increasing aeration, by stimulating microorganisms, by providing specific compounds, or by providing enhanced habitat and food diversity resulting in an enhanced diversity of soil fauna (Hättenschwiler et al. 2005).

Litter moisture is important for the activity of decomposers, particularly microbial decomposers (Swift et al. 1979). The physical structure of litter markedly affects its capacity to retain water (Makkonen et al. 2013). Wardle et al. (2003) found a positive effect of litters of high water-holding capacity (feather mosses or lichens) on the decomposition of litters of low water holding capacity. Makkonen et al. (2013) emphasized the context dependency of this phenomenon; litters of high water-holding capacity are only beneficial to litters of low water-holding capacity under conditions of limited moisture.

Mixed litters may complement each other in term of food quality for macro-detritivores, mesofauna, and microbes and increased food quality may result in increased rates of detritivory and activities of mesofauna and microbes that result in decomposition (Vos et al. 2013). Litter mixtures may also increase habitat heterogeneity for both fauna and microbes, resulting in their increased diversity (Blair et al. 1990, Hansen and Coleman 1998, Kaneko and Salamanca 1999, Hansen 2000, Wardle et al. 2006). While some studies suggest that increasing diversity of microbial and faunal communities contribute to non-additive decomposition (Blair et al. 1990, Kaneko and Salamanca 1999, Chapman et al. 2013), others have reported a weak impact or no impact of diversity (Wardle et al. 2006, Lummer et al. 2012). Limited impacts of enhanced biodiversity of litter organisms may be due to high functional redundancy among members of the

community, resulting in only limited effects of increasing taxonomic diversity on decomposition rate (Cleveland et al. 2014; Schimel and Gulledge 1998).

DECOMPOSER ORGANISMS

Microbes

The decomposition of plant litter compounds is performed largely by soil microbes, especially by fungi (Swift et al. 1979). This is because decomposition usually requires extracellular enzymes to break down large organic molecules and fungi (Berg and McClaugherty 2014), in general, produce a wider range of extracellular enzymes than bacteria (Romaní et al. 2006). Because the activities of these extracellular enzymes have a direct influence on decomposition, they are often correlated with litter decomposition rates. For example, Saiya-Cork et al. (2002) found that N addition increased decomposing enzyme activities and litter decomposition rate.

Microbial respiration rates have also been used to estimate litter decomposition rates (O'Connell 1990). However, this method cannot correctly reflect decomposition rates when labile C is added because the portion of evolved CO_2 due to metabolism of the labile C leads to an overestimation of the rate of resident organic matter decomposition (Boberg et al. 2008, Chigineva et al. 2009).

Soil fauna

While microorganisms are usually the primary decomposers of litter, the soil fauna is also a contributor (Swift et al. 1979). Its major contribution is via their interactions with microorganisms (Heneghan et al. 1999). Microarthropods, for example, increase the surface area

for microbial colonization (Seastedt 1984, Berg and McClaugherty 2014). Soil animals may also affect decomposition by controlling the composition of microbial communities through selective feeding (Elkins and Whitford 1982).

CONSIDERATIONS FOR A UNIFYING CONCEPTUAL MODEL

Based on the aforementioned principles, I propose that positive non-additive decomposition only occurs when one litter increases the activity of decomposer organisms in another litter either by improving its physical or chemical environment. There are four important points I need to consider with respect to this model.

First, either soil microbes or animals may contribute to positive non-additive decomposition. Litter mixtures may benefit microbes mainly by improving the availability of water, C, N, and other nutrients, any of which could be limiting resources. Litter mixtures may benefit soil animals by increasing habitat heterogeneity or providing complementary resources.

Second, the concentration of a resource is not the same as its availability. For example, the decomposition rate of litter with low N concentration is not always limited by N because it may be more limited by C availability. Only if one knows the actual limiting resources of the two litters can one correctly predict the decomposition rate of their mixture.

Third, labile C may have a range of effects on the decomposition of litter depending on its concentration. Even when litter decomposition is limited by C, the addition of labile C in large concentrations may lead to the cessation of enzyme production by decomposer microorganisms as they temporarily focus on metabolizing the labile C, the case I refer to as substitution. Only when additional labile C is given in small concentrations (still limiting) will

the microbes use this to increase enzyme production, leading to increased litter decomposition (priming).

Fourth, external sources of resources that limit microbial activity in one litter could come from a second litter type or from the soil. When decomposer microbes receive a limiting resource from an external source, microbial activity increases and this may lead to increased decomposition rate.

CONCLUSIONS

At the present time, it is very difficult to predict the rate of decomposition of mixed litters. However, by considering the four important points above, I believe one can begin to predict mixed litter decomposition rates.

CHAPTER 2: The role of microarthropods and microorganisms in positive, non-additive decomposition

INTRODUCTION

Litter decomposition is an essential ecosystem process. It results in the cycling of nutrients (Swift et al. 1979). It also transforms the relatively labile organic matter of litter into more stable forms referred to as soil organic matter, SOM. Some of this transformation occurs via the production of recalcitrant microbial compounds (Bird et al. 2008, Mambelli et al. 2011, Cotrufo et al. 2013). This conversion of labile to stable forms of organic material is important for two major reasons. First, the concentration of stable SOM determines soil fertility and tilth, so it is a key indicator of agricultural sustainability (Reeves 1997). Second, soils contain the largest terrestrial pool of organic C, mainly in the form of SOM, and a small change in its size can have a large effect on atmospheric CO_2 concentrations (Jobbágy and Jackson 2000). Therefore, understanding the factors that control litter decomposition is of great importance.

While climate is the most important single factor determining the rate of litter decomposition on a global scale, for a given climate much of the variation in decomposition can be explained by variation in litter quality (Aerts 1997). Litter quality, including chemistry and physical properties, is an important factor in litter decomposition because it determines the composition of microarthropod communities (Gergócs and Hufnagel 2016) that may contribute significantly to decomposition by fragmenting litter or by feeding on decomposer fungi (Seastedt 1984, Kampichler and Bruckner 2009), and because it influences the activities of decomposer microorganisms (Šnajdr et al. 2011, Talbot and Treseder 2012), which produce the enzymes that hydrolyze and oxidize litter compounds (Sinsabaugh and Moorhead 1994).

Understanding the role of litter quality in the decomposition of a single litter type is relatively straightforward. But litter quality varies with the plant species producing the litter and with litter age, and litter nearly always decomposes in heterogeneous combinations of litter type and age (Fyles and Fyles 1993). Moreover, new combinations of litter types are proliferating as plant communities are altered by climate change, by biological invasion and by novel crop rotations and intercropping strategies. It is difficult to predict decomposition rates of heterogeneous mixtures of litter. One litter type may have no effect on another, it may have a positive effect, or it may have a negative effect (McTiernan et al. 1997, Gartner and Cardon 2004).

We are particularly interested in the causes of non-additive, mixed litter decomposition, especially when the proximity of one litter type increases the decomposition of a second litter type. Several mechanisms have been proposed to explain this, but one of the most frequently proposed is that limiting resources are supplied by one litter type to the organisms decomposing the other litter type (Gartner and Cardon 2004) via fungal translocation (Boberg et al. 2014). These limiting resources include organic C compounds (Hallam and Bartholomew 1953), P (Koide and Shumway 2000), and N (Taylor et al. 1989, Schimel and Weintraub 2003, Schimel and Bennett 2004).

If limiting resources are translocated from one litter type to another, then one expects to find an elevated microbial activity or an elevated microarthropod abundance in the second as a consequence of its proximity to the former. Although some studies have investigated this (Bardgett and Shine 1999, Kaneko and Salamanca 1999, Wardle et al. 2006), it is still not well understood (Wardle et al. 2006). Therefore, I set out to determine whether microarthropod abundance or the activities of decomposition enzymes (as a proxy for microbial activity) were

increased in one litter type whose decomposition was increased by proximity to another litter type. We chose oat straw and clover as the mixture because both are common crops in the U.S.A. and because preliminary experiments demonstrated a significantly increased decomposition rate of oat straw when mixed with clover.

MATERIALS AND METHODS

Litter collection

Oats (*Avena sativa* L.) and red clover (*Trifolium pretense* L.), both planted in the fall of 2013, were harvested by hand on 10 May 2014 from an experimental farm at the Pennsylvania State University, State College, PA, USA. The harvested oat material (stems) was dead at harvest, whereas the clover was live. The materials were placed in paper bags and dried at 65˚C, then maintained at room temperature at Brigham Young University, UT, USA.

The total N contents of the litters were analyzed with a Combustion - Elementar Vario Max N/C Analyzer. Initial litter P, K, Ca, Mg, S, Na, Mn, Fe, Cu, B, Al, and Zn content were determined by inductively-coupled plasma (ICP) emissions spectrometry following digestion in nitric acid. All the measurements of litter nutrient quality were conducted at Agricultural Analytical Services Lab, Pennsylvania State University, State College, PA, USA.

Microarthropod activity experiments

We conducted two experiments concurrently. Experiment 1 addressed whether microarthropod abundance could account any effect of clover on oat straw decomposition. Experiment 2 addressed whether microarthropod abundance could account for any effect of oat

straw on clover decomposition. Each experiment was a randomized complete block design with six replicates and insecticide and litter combination (single vs. mixed) as main effects.

Mesh bags (7 cm x 8 cm) were constructed from window screen material (PVC-coated nylon mesh, 1.5 mm mesh size) using a heat sealer. Dried oat straw litter was cut into 1.5 cm pieces and mixed thoroughly. Dried clover litter was mixed thoroughly. Each litter type was used to fill 24 mesh bags each. Precautions were taken to avoid crushing the fragile clover litter into pieces small enough to pass through the mesh. Each mesh bag was filled with 1 ± 0.05 g of either oat straw or clover litter. Both oat straw and clover mesh bags were evenly split into insecticide-treated or untreated treatments. Insecticide-treated bags were saturated for 4 h in a 20 ppm solution of Fuse termiticide/insecticide, a combination of imidacloprid and fipronil in order to drastically reduce the abundance of microarthropods (Hainzl et al. 1998, Reynolds 2008, Hayasaka et al. 2012). Untreated bags were saturated for 4 h in distilled water. In either case, excess liquid was allowed to drain to produce the litter at full saturation. Approximately 3 g liquid remained in each sample. For the mixed litters, one oat straw litterbag and one clover litterbag were stapled together, and the clover mesh bag was on top of the oat straw mesh bag to promote the movement of microarthropods from the soil into oat straw on their way to the clover. Single litters consisted of a single oat straw mesh bag (Experiment 1) or a single clover mesh bag (Experiment 2).

Soil was collected from an agricultural field previously used to grow maize in Springville, UT, USA. We intentionally chose a soil from an agricultural system so the microarthropods would be similar to those from the system that was the source of the litter. Soil was collected from the top 8 cm. Large stones and other debris were removed, and the soil was thoroughly mixed. Then a 5 cm layer of this soil was pre-incubated at field capacity in each of

six plastic containers (52 cm x 38 cm x 14 cm high). Each container contained one replicate of each of the 4 treatment combinations (insecticide/control x single/mixed) from each experiment. The mesh bags of a given replicate were laid in random positions on the soil surface in each container. The distance between mesh bags was 12-18 cm, which I assumed was large enough to minimize the effect of the insecticides on untreated mesh bags. The sealed, plastic containers were covered with black plastic bags to protect from light and kept in the lab at room temperature (23 ± 0.5˚C).

Litter from the mesh bags was harvested after 25 d of incubation. Microarthropods were extracted for 24 h using Berlese funnels. Among the two experiments, there were 48 mesh bags but there were only 8 funnels. Thus, microarthropods were collected from a single replicate of both experiments at a time. The remaining mesh bags were stored at 4°C prior to extraction. To prevent the escape of microarthropods and loss of water while refrigerated, the mesh bags were placed individually into plastic bags. Even for the mixed litter treatments, oat straw and clover litters were stored in separate bags and microarthropods were collected separately from each bag. After extraction, microarthropods were immediately counted. Densities were expressed as numbers of individuals g^{-1} of litter remaining. Following microarthropod collection, intact mesh bags were oven-dried at 65˚C, and litter samples were weighed in order to calculate the decomposition rate.

Microbial activity experiment

This experiment involved two treatments, oat straw by itself and oat straw with clover. There were eight replicates for a total of 16 units. Mesh bags, as above, were filled in the same way as for the microarthropod experiments. Oat straw and clover litterbags were saturated in

purified water separately for 4h. Excess water was allowed to drain to produce the litter at full saturation. When oat straw was incubated with clover, oat straw mesh bags were stapled to and on top of the clover mesh bags to prevent the movement of liquid from clover to oat straw litter due to gravity. Each sample was incubated in a petri dish and all the petri dishes were randomly placed in one large plastic container to maintain a high humidity with damp paper towels placed in the bottom of the container. The container was kept in the laboratory at room temperature (23 ± 0.5˚C).

Litters were harvested after 28 days of incubation. Each litter was aliquoted into two subsamples, one of which was used for enzyme analyses, the other of which was used to calculate the decomposition rate. The first subsamples were ground with mortar and pestle in liquid N to a fine powder, and stored at -20˚C. The second subsamples were weighed, oven-dried at 65˚C, and weighed again in order to calculate decomposition rate.

Enzyme activities were used as proxies for microbial activity. The concentration of lignin in grass litter is low (Vogel 2008), so enzymes involved in cellulose hydrolysis are of greatest interest. The activities of both cellulases and xylanase are important for oat straw decomposition. Consequently, I measured the activity of β-glucosidase (BG, EC 3.2.1.21), cellobiohydrolase (CBH, EC 3.2.1.91), and β-xylosidase (BX, EC 3.2.1.37) with assay techniques modified from Peoples and Koide (2012). Activities of these three enzymes were measured fluorometrically using as substrates 4-methylumbelliferyl-β-D-glucopyranoside (MUB-GP, Cayman Chemical), 4-methylumbelliferyl-β-D-cellobioside (MUB-CB, P212121), and 4-methylumbelliferyl-β-D-xylopyranoside (MUB-XP, Carbosynth), respectively.

For each sample 0.5 g (fresh weight) ground litter was added to 50 mL distilled water and homogenized by hand for 30 s. Because enzyme activity is pH sensitive, I used water rather than

buffer so the solution remained at the pH of the litter. Then, 1 mL of litter homogenate was immediately transferred to a 2 mL microfuge tube. The end of pipette tips was clipped to accommodate litter particles. The MUB-linked substrate (0.5mL of 200μM), was added to the homogenates and the microfuge tubes were placed horizontally on a mixer at low speed for the various periods of incubation (30 min for MUB-GP, 45 min for MUB-CB, 45 min for MUB-XP). Upon completion of the incubation, 0.5 mL of 50 mM sodium hydroxide (NaOH) was added to stop the reaction, bringing the total volume in each tube to 2 mL. Tubes were then centrifuged at 10,000 × g for 1 min. A 200 μL aliquot of supernatant from each sample was added to each of 8 wells of a black, polystyrene 96-well microplate to yield eight analytical replicates per experimental replicate. Four additional columns (each consisting of 8 replicate wells) were filled in the following order as in DeForest (2009): MUB standard wells contained 100 μL water, 50 μL of 10 μM β-methylumbelliferone (MUB, Sigma-Aldrich) and 50 μL of 50 mM NaOH. The substrate blank wells contained 100 μL water, 50 μL of 200 μM substrate and 50 μL of 50 mM NaOH. The sample autofluorescence blank wells contained 50 μL water, 100 μL of sample supernatant and 50 μL of 50 mM NaOH. The quenching control wells contained 100 μL of sample supernatant, 50 μL of 10 μM MUB and 50 μL of 50 mM NaOH. Fluorescence was determined using a Biotek Synergy HT spectrophotometer with a 360 nm excitation filter and 460 nm emission filter. Enzyme activities were calculated from average fluorescences of the eight analytical replicates by the equations in DeForest (2009).

Data analysis

For the microarthropod experiments (oat straw and clover separately), data were subjected to analysis of variance with the block, insecticide treatment and litter combination plus

the interaction between insecticide treatment, and litter combination as factors. For the microbial activity experiment, data were subjected to analysis of variance with litter combination the sole factor. All analyses and post-hoc tests were conducted in the R software environment (R Core Team 2013).

RESULTS

The initial litter quality, as measured by initial concentrations of macro-elements (N, P, K, Ca, Mg, and S) and micro-elements (Mn, Fe, Cu, B, Al, Zn and Na), greatly differ between oat straw and clover litter (Table 2.1). The concentrations of N, P, K, Ca, Mg, S, Mn, Cu, B, and Zn were higher in clover litter, and the concentrations of Fe, Al, and Na were higher in oat straw litter.

Table 2.1. Initial chemical compositions of oat straw and clover litter. Data are means ± SEM, n = 2.

Macro elements	N (%)	P	K	Ca	Mg	S	
Oat straw	0.813±0.054	0.097±0.007	0.285±0.092	0.182±0.018	0.082±0.000	0.081±0.004	
Clover	4.67±0.173	0.379±0.005	3.52±0.158	1.36±0.061	0.307±0.001	0.238±0.007	
Micro elements	Mn (ppm)	Fe	Cu	B	Al	Zn	Na
Oat straw	71.2±3.10	259±103	5.29±1.50	9.97±0.016	112±54.1	17.2±1.32	47.8±1.92
Clover	77.6±1.47	146±17.1	15.9±0.632	20.5±0.045	59.9±14.0	32.7±3.12	9.96±0.022

Micro-arthropod activity experiments

For both oat straw and clover litter there were significant litter combination effects (single vs. mixed) but no significant insecticide effects (control vs. insecticide) on mean decomposition rates (Table 2.2, Figure 2.1). The interaction between insecticide and litter combination for both oat straw and clover decomposition was not significant (Table 2.2). Oat straw decomposition significantly increased by an average of 34.6% when no insecticide was applied and by an average of 33.8% when it was applied when oat straw was combined with clover as compared to oat straw alone (Figure 2.1a). Clover decomposition significantly increased by an average of 4.3% when no insecticide was applied and by an average of 4.0% when it was applied when clover was combined with oat straw as compared to clover alone (Figure 2.1b).

Table 2.2. Results of two-way analysis of variance (ANOVA) for decomposition of oat straw and clover, with block, insecticide treatment (I), litter combination (C) and the interaction between insecticide treatment and litter combination as explanatory variables.

Source of variation	Oat straw					Clover				
	df	SS	MS	*F*	*P*	df	SS	MS	*F*	*P*
Model	8	57.9	7.24	10.5	**<.0001**	8	13.3	1.657	4.50	**0.0059**
Error	15	10.3	0.689			15	5.52	0.368		
Total	23	68.2				23	18.8			
Effects test										
Block	5	6.68		1.94	0.147	5	3.95		2.15	0.115
Insecticide (I)	1	0.022		0.0321	0.860	1	0.0266		0.0723	0.792
Combination (C)	1	51.2		74.3	**<.0001**	1	9.27		25.2	**0.0002**
I × C	1	0.0077		0.0112	0.917	1	0.0068		0.0186	0.893

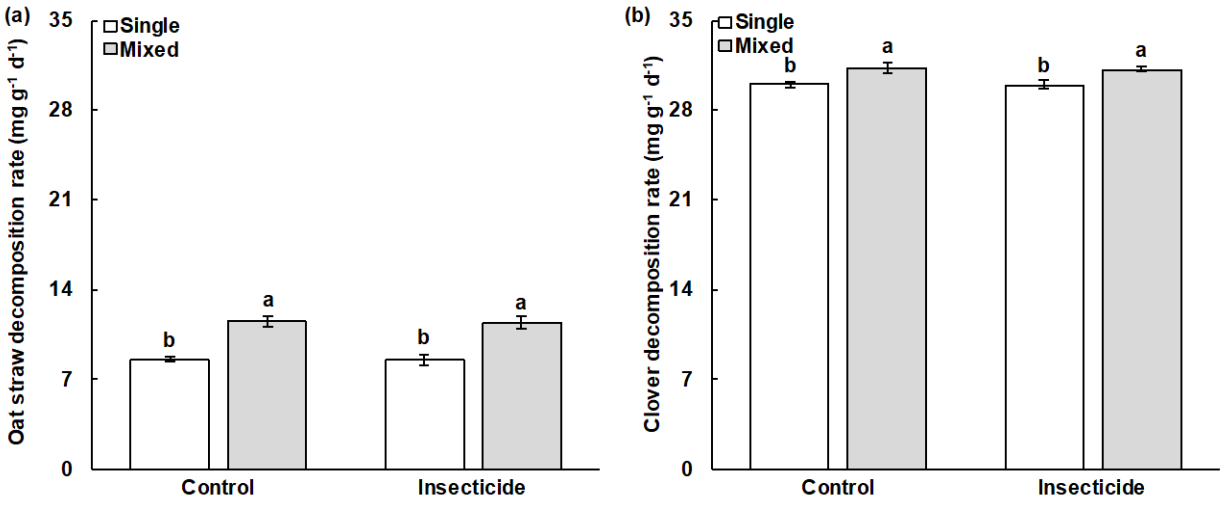

Figure 2.1. The effect of insecticide and litter combination on mean decomposition rate of oat straw (a) and clover (b). Vertical lines are ± 1 SEM.

For both oat straw and clover litter, mites account for over 99% of the microarthropods extracted from litter (Table 2.3). Among all the samples, I detected only 3 collembola, all of them occurred in oat straw litter. There were two mite genera in this study; Sancassania is saprophagous while Gaeolaelaps is predaceous. Sancassania dominated the mite community. Only a few Gaeolaelaps mites were found in the control treatment (no insecticide) for both oat straw and clover (Table 2.3).

Table 2.3. Abundance of various groups of microarthropods extracted from oat straw and clover litter. Data are means ± SEM, n = 6.

		Oat straw			Clover		
Treatment	Combination	*Sancassania*	*Gaeolaelaps*	Collembola	*Sancassania*	*Gaeolaelaps*	Collembola
Control	Single	686 ± 91.7	1.67 ± 0.617	0	590 ± 189	1.17 ± 0.707	0
	Mixed	650 ± 123	1.17 ± 0.471	0.500 ± 0.218	408 ± 101	0.500 ± 0.321	0
Insecticide	Single	34.2 ± 7.21	0	0	150 ± 36.9	0	0
	Mixed	119 ± 83.3	0	0	87.5 ± 48.7	0	0

For both oat straw and clover litter, insecticide significantly reduced microarthropod densities, but litter combination had no significant effect on microarthropod densities (Table 2.4, Figure 2.2). There was no significant interaction between insecticide and litter combination (Table 2.4). Therefore, for both oat straw and clover, the positive, non-additive decomposition was not associated with enhanced microarthropod density.

Table 2.4. Results of two-way analysis of variance (ANOVA) for microarthropod density in oat straw and in clover, with block, insecticide treatment (I), litter combination (C) and the interaction between insecticide treatment and litter combination as explanatory variables.

Source of variation	Oat straw					Clover				
	df	SS	MS	*F*	*P*	df	SS	MS	*F*	*P*
Model	8	$4.03x10^6$	$5.04x10^5$	7.77	**0.0004**	8	6.03	0.754	5.47	**0.0024**
Error	15	$9.73x10^5$	$6.48x10^4$			15	2.07	0.138		
Total	23	$5.00x10^6$				23	8.10			
Effects test										
Block	5	$3.04x10^5$		0.937	0.485	5	1.98		2.87	0.0517
Insecticide (I)	1	$3.68x10^6$		56.7	**<.0001**	1	3.35		24.3	**0.0002**
Combination (C)	1	$3.60x10^4$		0.556	0.468	1	0.364		2.64	0.125
I × C	1	$1.18x10^4$		0.181	0.676	1	0.339		2.46	0.138

Notes: For clover litter experiment, statistical analyses were performed on data that were log transformed.

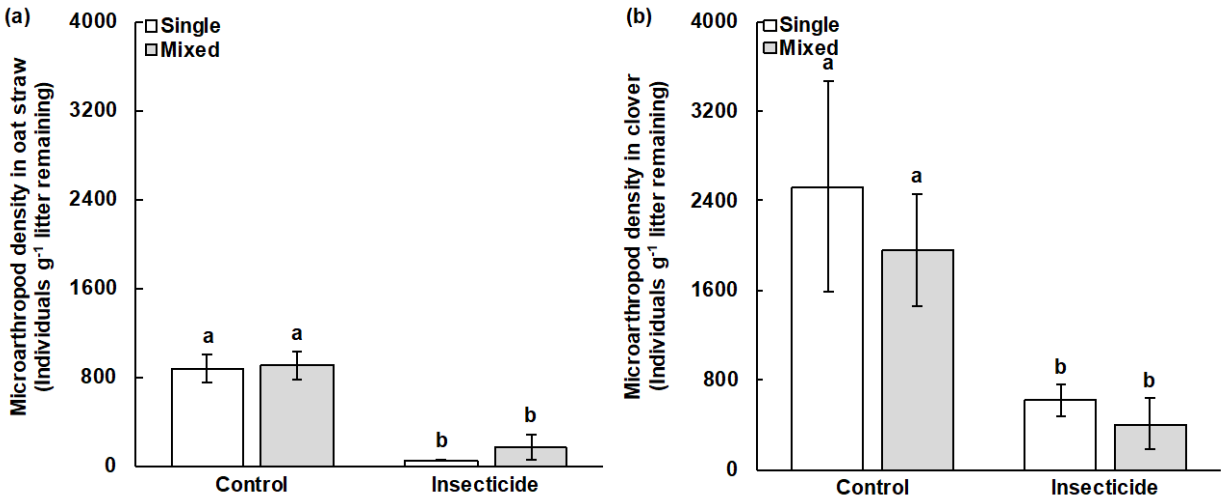

Figure 2.2. The effect of insecticide and litter combination (single vs. mixed) on mean microarthropod density in oat straw and (a) in clover (b). Vertical lines are ± 1 SEM.

Microbial activity experiment

Clover litter did increase the decomposition of oat straw (one-tailed t-test, $t_{14} = 5.41$, $P < 0.0001$, Figure 2.3a), during which β-glucosidase, cellobiohydrolase, and β-xylosidase activities were highest when oat straw litter was combined with clover, and significantly lower when oat straw litter was alone (β-glucosidase: one-tailed t-test, $t_{14} = 11.3$, $P < 0.0001$, cellobiohydrolase: one-tailed t-test, $t_{14} = 8.13$, $P < 0.0001$, β-xylosidase: one-tailed t-test, $t_{14} = 7.23$, $P < 0.0001$, Figure 2.3b), indicating that the presence of clover significantly increased cellulose hydrolytic activity in oat straw.

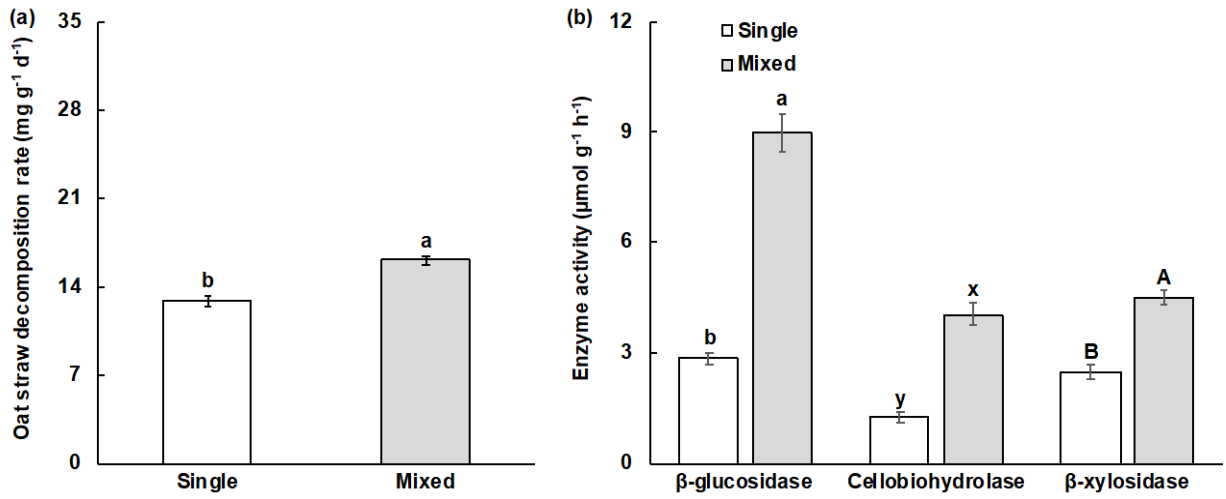

Figure 2.3. The effect of litter combination on mean decomposition rate of oat straw (a), and mean enzyme activity in oat straw (b). The enzymes are β-glucosidase, cellobiohydrolase and β-xylosidase. Vertical lines are ± 1 SEM. Different letters indicate significant ($P < 0.0001$) differences between oat straw by itself (Single) and when mixed with clover litter (Mixed) according to t-test.

DISCUSSION

Our goal was to determine the relative importance of alterations to microarthropod activity and microbial activity to the phenomenon of positive, non-additive decomposition. We found good evidence for positive non-additive decomposition in our system; the presence of clover litter stimulated the decomposition of oat straw. We assumed that clover litter could stimulate microarthropod abundance or microbial activity in the oat straw by providing limiting resources that were transferable from clover to oat straw by, for example, the saprotrophic fungi (Lummer et al. 2012, Montané et al. 2013, Ghasemi-Aghbash et al. 2016). Clover did, indeed, contain higher concentrations of several potentially limiting nutrients than clover, including N, P, K, Ca, Mg, S, Mn, Cu, B, and Zn. Reduced C compounds may also have been more available from clover litter than from oat straw due to the complex crosslinking of polysaccharides in grass cell walls (Vogel 2008).

The vast majority of the microarthropods in these two litter types were mites. Other than a total of 3 collembola, no other kinds of microarthropods were observed. Moreover, the vast majority of mites were saprophagous, so their abundance was expected to influence decomposition. Nevertheless, while microarthropod density was significantly higher in clover litter than in oat straw litter, microarthropod density in oat straw was not significantly affected by the presence of clover litter. Furthermore, insecticides significantly reduced the density of microarthropods in oat straw, they had no significant impact on oat straw decomposition. These results demonstrate that clover did not increase the decomposition rate of oat straw by enhancing microarthropod density in oat straw in this study. Our results were consistent with those of Jiang et al. (2013), who similarly concluded that non-additive decomposition was not due to changes in communities of soil fauna. Some other mechanism must have been responsible for the positive effect of clover litter on oat straw decomposition.

Microorganisms produce the enzymes that are ultimately responsible for the decomposition of litter (Sinsabaugh et al. 2008). β-glucosidase and cellobiohydrolase are responsible for the hydrolysis of β-1,4 glucoside bonds in cellulose. In the enzymatic hydrolysis of cellulose, cellobiohydrolase is responsible for the release of cellobiose, after which β-glucosidase hydrolyzes cellobiose to free glucose molecules (Yeoman et al. 2010). β-xylosidase hydrolyzes xylose from xylan, a type of hemicellulose (Bajpai 2014). The activities of these decomposition enzymes were significantly higher in oat straw in the presence of clover litter. It would appear, therefore, that enhanced enzyme activity in oat straw is one mechanism by which clover litter enhances the rate of decomposition of oat straw. We conclude, therefore, that in our clover/oat straw system, microorganism activity in the form of enzyme production was more important than alternations to microarthropod abundance of community structure. Nevertheless,

one can envision circumstances in which microarthropods could play a significant role in positive, non-additive decomposition, either when enzyme action is limited by litter surface area, or when microarthropods consume significant amounts of fungi or bacteria, affecting their activities.

The conditions under which I performed these experiments were somewhat artificial. In the laboratory, decomposition in our studies occurred at constant temperature and moisture, which is unlike the conditions in most field settings. Our findings may, therefore, not be generalizable to all field settings. Nevertheless, the litters were not sterile, and non-sterile soil was used as a source of soil microarthropods. Therefore, the conditions were not entirely unnatural.

If positive, non-additive decomposition occurs as a consequence of the stimulation of microbial activity in the more recalcitrant litter type litter in the presence of a more labile litter type, as I have shown, then it is logical to hypothesize that this should occur when a limiting resource or set of resources are supplied by the second to the former. This could be mineral nutrients, reduced C (energy source) or moisture. The characterization of that limiting resource is the subject of the next chapter.

CHAPTER 3: The role of C and mineral nutrient transfer in positive, non-additive decomposition

INTRODUCTION

Litter decomposition is an essential ecosystem process. It results in the cycling of nutrients (Swift et al. 1979). It also transforms the relatively labile organic matter of litter into more stable forms referred to as soil organic matter, SOM. Some of this transformation occurs via the production of recalcitrant microbial compounds (Bird et al. 2008, Mambelli et al. 2011, Cotrufo et al. 2013). This conversion of labile to stable forms of organic material is important for two major reasons. First, the concentration of stable SOM determines soil fertility and tilth, so it is a key indicator of agricultural sustainability (Reeves 1997). Second, soils contain the largest terrestrial pool of organic C, mainly in the form of SOM, and a small change in its size can have a large effect on atmospheric CO_2 concentrations (Jobbágy and Jackson 2000).

In both agricultural and natural systems, most plant litter is of mixed quality, which varies with the plant species producing the litter and with litter age. Thus, litter nearly always decomposes in heterogeneous combinations of litter type and age (Fyles and Fyles 1993). It is difficult to predict decomposition rates of heterogeneous mixtures of litter. One litter type may have no effect on another, it may have a positive effect, or it may have a negative effect (McTiernan et al. 1997, Gartner and Cardon 2004). The latter two (the positive effect and negative effect) are referred to as non-additive decomposition. We are primarily interested in understanding the mechanisms responsible for positive, non-additive decomposition as it has previously been impossible to predict.

Using an oat straw – clover litter system, I previously showed that the positive effect of clover litter on oat straw decomposition was associated with an increase in the activities of

various enzymes involved in litter decomposition in the oat straw, presumably due to increased microbial activity, but was not associated with a change in microarthropod abundance. In this contribution, I further investigate the mechanisms leading to positive, non-additive decomposition by determining the resources that limit microbial activity in oat straw.

We tested six hypotheses in this study: moisture limits microbial activity in oat straw; N limits microbial activity in oat straw; C limits microbial activity in oat straw; C and N together limit microbial activity in oat straw; C, N and other mineral nutrients together limit microbial activity in oat straw; soil can supply limiting mineral nutrients to microbes to decomposing oat straw.

MATERIALS AND METHODS

Litter collection

Oats (*Avena sativa* L.) and red clover (*Trifolium pretense* L.), both planted in the fall of 2014, were harvested by hand on 19 May 2015 from an experimental farm at the Pennsylvania State University, State College, PA, USA. The harvested oat material (stems) was dead at harvest, whereas the clover was live. The materials were placed in paper bags and dried at 65˚C, then maintained at room temperature at Brigham Young University, UT, USA.

Experiment 1. Moisture limits microbial activity in oat straw

This experiment had two treatments, saturated oat straw litter, and saturated oat straw litter with an additional source of water to determine whether the positive effect of clover on oat straw decomposition could be due to the water supplied by clover. Additional water was supplied via an inert (fiberglass) pad within a mesh bag. Mesh bags (7×8 cm) were constructed from

window screen material (PVC-coated nylon mesh, 1.5 mm mesh size) using a heat sealer. Dried oat straw litter was cut into 1.5 cm pieces and mixed thoroughly, then used to fill 10 mesh bags with 1 ± 0.05 g of oat straw. Fiberglass pads were placed in 5 mesh bags of the same construction. Oat straw mesh bags were saturated in purified water for 4 h. Excess water was allowed to drain to produce the litter at full saturation. Approximately 3 g water remained in each sample. Each fiberglass pad held 3.5 mL purified water (approximately the same amount of water held by 1 g dry clover litter).

Oat straw mesh bags were incubated either by themselves or with a saturated fiberglass pad. There were five replicates per treatment. When oat straw was incubated with a fiberglass pad, the oat straw mesh bag was stapled to and on top of the mesh bag holding the fiberglass pad in order to prevent water moving from fiberglass pad to the oat straw litter due to gravity. Each sample was incubated in a petri dish and all the petri dishes were randomly placed in one large plastic container to maintain a high humidity with damp paper towels placed in the bottom of the container. The container was kept in the laboratory at room temperature (23 ± 0.5˚C). Litters were harvested after 25 days of incubation. Intact mesh bags were oven-dried at 65˚C and litter samples were weighed to calculate decomposition rate.

Experiment 2. N limits microbial activity in oat straw.

This experiment had four treatments: 1) oat straw with clover, 2) oat straw with additional water, 3) oat straw with a solution containing 2 mg N as NH_4Cl g^{-1} dry weight of litter (equal to 25% of the total N concentration in oat straw), and 4) oat straw with a solution containing 4 mg N as NH_4Cl g^{-1} dry weight of litter (equal to 50% of the total N concentration in oat straw). The additional water and the NH_4Cl solutions were added to a fiberglass pad as in the

previous experiment. All the fiberglass pads contained the same amount of liquid as when 1 g dry weight of clover litter is fully saturated (approximately 3.5 mL). Mesh bags were constructed as in the previous experiment. Each treatment was replicated six times. Thus, there were 24 oat straw mesh bags, 6 clover mesh bags, 6 mesh bags containing fiberglass pads with 2 mg N as NH_4Cl g^{-1} dry litter, and 6 mesh bags containing fiberglass pads with 4 mg N as NH_4Cl g^{-1} dry litter.

When oat straw was incubated with a fiberglass pad or with clover, oat straw mesh bags were stapled to and on top of the other mesh bag in order to prevent water moving from clover or fiberglass to the oat straw litter due to gravity. Each sample was incubated in a petri dish and all the petri dishes were placed in one large plastic container to maintain a high humidity with damp paper towels placed in the bottom of the container. The container was kept in the laboratory at room temperature (23 ± 0.5˚C). Litters were harvested after 25 days of incubation. Intact mesh bags were oven-dried at 65˚C and litter samples were weighed to calculate decomposition rate.

Experiment 3. C limits microbial activity in oat straw.

Experiment 3.1. The purpose of this experiment was to establish the optimum glucose concentrations for decomposition of oat straw. There were seven treatments: 1) oat straw with additional water, 2) oat straw with 50 µg C as glucose g^{-1} dry litter, 3) oat straw with 100 µg C as glucose g^{-1} dry litter, 4) oat straw with 200 µg C as glucose g^{-1} dry litter, 5) oat straw with 500 µg C as glucose g^{-1} dry litter, 6) oat straw with 1000 µg C as glucose g^{-1} dry litter, and 7) oat straw with 3500 µg C as glucose g^{-1} dry litter. Additional water or glucose solutions were added to fiberglass pads and all the fiberglass pads contained the same amount of liquid as when 1 g dry weight of clover litter is fully saturated (approximately 3.5 mL). Mesh bags were constructed

as in the previous experiment. Each treatment had 6 replicates. In order to prevent water moving from fiberglass to the oat straw litter due to gravity, oat straw mesh bags were stapled to and on top of the fiberglass mesh bags. Each sample was incubated in a petri dish and all the petri dishes were placed in one large plastic container to maintain a high humidity with damp paper towels placed in the bottom of the container. The container was kept in the laboratory at room temperature (23 ± 0.5˚C).

Litter bags were harvested after 7 days of incubation. Each litter sample was separated into two subsamples, one of which was used to calculate litter weight after decomposition and the other to determine enzyme activity. The enzyme subsamples were ground to a fine powder using mortar and pestle in liquid N. Cellobiohydrolase activity was measured as in Chapter 2. The other subsamples were weighed, oven-dried at 65˚C and weighed again in order to calculate remaining weight of oat straw.

Experiment 3.2. This experiment had four treatments: 1) oat straw with clover, 2) oat straw with additional water, 3) oat straw with 500 µg C as glucose g^{-1} dry litter, and 4) oat straw with 4000 µg C as glucose g^{-1} dry litter. Previous experiments indicated that cellobiohydrolase and beta-glucosidase activities in oat straw were correlated with oat straw decomposition rate (Figure 2.3), and the preliminary experiment showed that 500 µg C as glucose g^{-1} dry litter gave the maximum cellobiohydrolase activity in oat straw, with higher and lower concentrations of glucose producing significantly lower cellobiohydrolase activities (Figure 3.3). Additional water or glucose solutions were added to fiberglass pads and all the fiberglass pads contained the same amount of liquid as when 1 g dry weight of clover litter is fully saturated (approximately 3.5 mL). Mesh bags were constructed as in the previous experiment. Each treatment was replicated 24 times so that 8 replicates could be sampled on each of 3 occasions.

For all the treatments, oat straw mesh bags were stapled to and on top of the other mesh bag in order to prevent water moving from clover or fiberglass to the oat straw litter due to gravity. Each replicate was incubated in a petri dish and all the petri dishes were placed in one large plastic container to maintain a high humidity with damp paper towels placed in the bottom of the container. The container was kept in the laboratory at room temperature (23 ± 0.5˚C). On 0, 7, 14, 21 days of incubation, 400 µL of the appropriate glucose solutions were added to the fiberglass pads of the glucose treatments to add 500 or 4000 µg C as glucose g^{-1} dry litter on each occasion, and 400 µL water were added to the fiberglass pad of the additional water treatment and to the clover in the oat straw with clover treatment.

Mesh bags were harvested at 14, 21 and 28 days of incubation. Each litter sample was separated into two subsamples, one of which one was used to determining enzyme activity and the other to calculate decomposition rate. The enzyme subsamples were ground to a fine powder using mortar and pestle in liquid N. Cellobiohydrolase activity was measured as in Chapter 2. Other enzymes involved in decomposition (β-glucosidase and β-xylosidase) were not measured because the activities of the three are highly correlated to each other (Figure 2.4). The other subsamples were weighed, oven-dried at 65˚C and weighed again in order to calculate decomposition rate.

Experiment 4. C and N together limit microbial activity in oat straw.

This experiment had four treatments: 1) oat straw with clover, 2) oat straw with additional water, 3) oat straw with 500 µg C as glucose g^{-1} dry litter, and 4) oat straw with 500 µg C as glucose g^{-1} dry litter plus 137 µg N as NH_4Cl g^{-1} dry litter. The C: N ratio of the latter mixture, 3.65, is based on that of plant peptone, a hydrolysate of plant protein, which, according

to our analyses, possesses a C: N ratio of 3.65. The water, glucose solution or glucose + NH_4Cl solution were added to fiberglass pads as in previous experiments, and all the fiberglass pads contained the same amount of liquid as when 1 g dry weight of clover litter is fully saturated (approximately 3.5 mL). Mesh bags were constructed as in the previous experiment. Each treatment was replicated eight times.

For all the treatments, oat straw mesh bags were stapled to and on top of the other mesh bag in order to prevent water moving from clover or fiberglass to the oat straw litter due to gravity. Each replicate was incubated in a petri dish and all the petri dishes were placed in one large plastic container to maintain a high humidity with damp paper towels placed in the bottom of the container. The container was kept in the laboratory at room temperature (23 ± 0.5˚C).

On 0, 7, 14, 21 days of incubation, 400 µL of appropriate solutions were added to the pads of treatment 3 in order to add 500 µg C as glucose g^{-1} dry litter, and 400 µL of appropriate solutions were added to the pads of treatment 4 in order to add 500 µg C as glucose g^{-1} dry litter and 137 µg N as NH_4Cl g^{-1} dry litter. 400 µL water were added on each occasion to the fiberglass pads in treatment 2 and to the clover in treatment 1.

Mesh bags were harvested after 28 days of incubation. Each litter sample was separated into two subsamples, one of which one was used to determining enzyme activity and the other to calculate decomposition rate. The enzyme subsamples were ground to a fine powder using mortar and pestle in liquid N. Cellobiohydrolase activity was measured as in Chapter 2. Other enzymes involved in decomposition (β-glucosidase and β-xylosidase) were not measured because the activities of the three are highly correlated to each other (Figure 2.4). The other subsamples were weighed, oven-dried at 65˚C and weighed again in order to calculate decomposition rate.

Experiment 5. C, N and other mineral nutrients together limit microbial activity in oat straw.

This experiment had three treatments: 1) oat straw with additional water, 2) oat straw with clover, and 3) oat straw with 500 µg C as glucose g^{-1} dry litter plus 137 µg N as NH_4Cl g^{-1} dry litter and all other mineral nutrients supplied in the same ratio as in Hoagland solution (Hoagland and Arnon 1950). Thus, for treatment 3, in addition to the NH_4Cl, the other salts included KH_2PO_4, KCl, $CaCl_2$, $MgSO_4$, FeNaEDTA, H_3BO_3, $MnSO_4$-H_2O, $ZnSO_4$-$7H_2O$, $CuSO_4$-$5H_2O$ and $(NH_4)_6Mo_7)_{24}$-$4H_2O$, supplying (in µg g^{-1} dry litter) 137, 20.2, 152.1, 130, 31.2, 41.6, 3.23, 1.3, 0.16, 0.16, 0.1, 0.02 and 0.02 for N, P, K, Ca, Mg, S, Fe, Cl, B, Mn, Zn, Cu and Mo, respectively. The additional water and the nutrient solution were initially added to a fiberglass pad as in the previous experiment. All the fiberglass pads contained the same amount of liquid as when 1 g dry weight of clover litter is fully saturated (approximately 3.5 mL). Mesh bags were constructed as in the previous experiment. Each treatment was replicated eight times.

For all the treatments, oat straw mesh bags were stapled to and on top of the other mesh bag in order to prevent water moving from clover or fiberglass to the oat straw litter due to gravity. Each replicate was incubated in a petri dish and all the petri dishes were placed in one large plastic container to maintain a high humidity with damp paper towels placed in the bottom of the container. The container was kept in the laboratory at room temperature (23 ± 0.5˚C).

On 0, 7, 14, 21 days of incubation, 400 µL of appropriate solution was added to the pads of treatment 3 in order to add 500 µg C as glucose g^{-1} dry litter, 137 µg N as NH_4Cl g^{-1} dry litter, and all the other nutrient minerals in their respective concentrations indicated above. 400 µL water were added on each occasion to the fiberglass pads in treatment 1 and to the clover in treatment 2.

Mesh bags were harvested after 28 days of incubation. Each litter sample was separated into two subsamples, one of which one was used to determining enzyme activity and the other to calculate decomposition rate. The enzyme subsamples were ground to a fine powder using mortar and pestle in liquid N. Cellobiohydrolase activity was measured as in Chapter 2. Other enzymes involved in decomposition (β-glucosidase and β-xylosidase) were not measured because the activities of the three are highly correlated to each other (Figure 2.4). The other subsamples were weighed, oven-dried at 65˚C and weighed again in order to calculate decomposition rate.

Experiment 6. Soil can supply limiting mineral nutrients to microbes to decomposing oat straw.

This experiment had four treatments: 1) oat straw with additional water, 2) oat straw with clover, 3) oat straw with 500 µg C as glucose g^{-1} dry litter and 137 µg N as NH_4Cl g^{-1} dry litter, and 4) oat straw with 500 µg C as glucose g^{-1} dry litter and 137 µg N as NH_4Cl g^{-1} dry litter overlying soil. The additional water and the nutrient solution were initially added to a fiberglass pad in a mesh bag below the mesh bag containing the oat straw as in the previous experiment. All fiberglass pads contained the same amount of liquid as when 1 g dry weight of clover litter is fully saturated (approximately 3.5 mL). Mesh bags were constructed as in the previous experiment. Each treatment was replicated six times.

The soil had been collected from a nearby agricultural field which had been planted to maize, then air dried at room temperature. Large stones and other debris were removed, then the soil was thoroughly mixed. To eliminate any effects of live soil organisms, dry soil was sterilized in an autoclave (120˚C, 20 min, 2x). For treatment 3, a thin layer (0.5 cm) of the sterilized soil

(50 g) was placed in the petri dishes, rewet soil to field capacity, and the mesh bags were placed on top of the soil.

For all the treatments, oat straw mesh bags were stapled to and on top of the other mesh bag in order to prevent water moving from clover or fiberglass to the oat straw litter due to gravity. Each replicate was incubated in a petri dish and all the petri dishes were placed in one large plastic container to maintain a high humidity with damp paper towels placed in the bottom of the container. The container was kept in the laboratory at room temperature (23 ± 0.5˚C).

On 0, 7, 14, 21 days of incubation, 400 µL of appropriate solution was added to the pads of treatments 3 and 4 in order to add 500 µg C as glucose g^{-1} dry litter and 137 µg N as NH_4Cl g^{-1} dry litter. 400 µL water were added on each occasion to the fiberglass pads in treatment 1 and to the clover in treatment 2.

Mesh bags were harvested after 28 days of incubation. Each litter sample was separated into two subsamples, one of which one was used to determining enzyme activity and the other to calculate decomposition rate. The enzyme subsamples were ground to a fine powder using mortar and pestle in liquid N. Cellobiohydrolase activity was measured as in Chapter 2. Other enzymes involved in decomposition (β-glucosidase and β-xylosidase) were not measured because the activities of the three are highly correlated to each other (Figure 2.4). The other subsamples were weighed, oven-dried at 65˚C and weighed again in order to calculate decomposition rate.

Data analysis

For all analyses of variance, data were transformed, as necessary, to satisfy the assumptions of analysis of variance including normality and homogeneity of variance. Significant differences among treatment means in oat straw decomposition rate or

cellobiohydrolase activity was determined by the least significant difference (LSD, p-value < 0.05) method. All analyses and post-hoc tests were conducted in the R software environment (R Core Team 2013).

RESULTS

Experiment 1. Moisture limits microbial activity in oat straw.

There was no significant increase of oat straw decomposition rate by amending oat straw at field capacity with an additional source of water during the course of 25 d experiment (one-tailed t-test, $t_8 = 0.54$, $P = 0.699$, Figure 3.1).

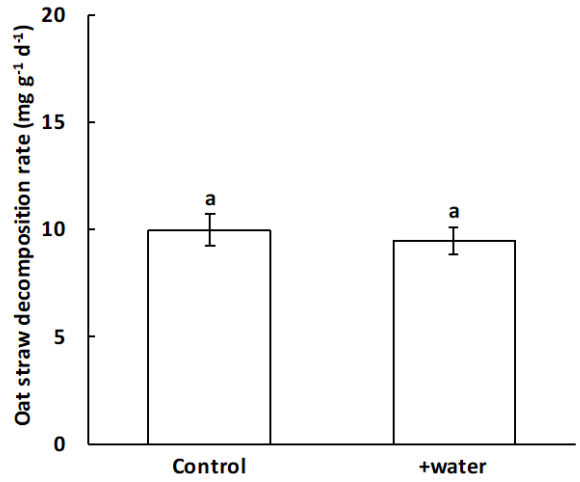

Figure 3.1. The effect of additional water on mean decomposition rate of oat straw. Control = oat straw by itself; + water = oat straw alone but with additional water. Vertical lines are ± 1 SEM.

Experiment 2. N limits microbial activity in oat straw.

After 25 d of incubation, there was a significant difference in mean oat straw decomposition rate among treatments (Table 3.1, Figure 3.2). Clover significantly increased oat straw decomposition rate relative to oat straw alone, but both high and low NH_4Cl concentration treatment had no significant effect on oat straw decomposition rate compared with the single oat

straw treatment. Although the low NH_4Cl concentration treatment increased (not significantly) oat straw decomposition rate, the high NH_4Cl concentration treatment did not increase oat straw decomposition rate more than the low NH_4Cl concentration treatment, suggesting that N alone was not the limiting resource for oat straw decomposition.

Table 3.1. Results of one-way analysis of variance (ANOVA) for oat straw decomposition.

Source of variation	df	SS	MS	*F*	*P*
Treatment	3	56.0	18.7	12.7	**0.0002**
Residuals	16	23.5	1.47		
Total	19	79.5			

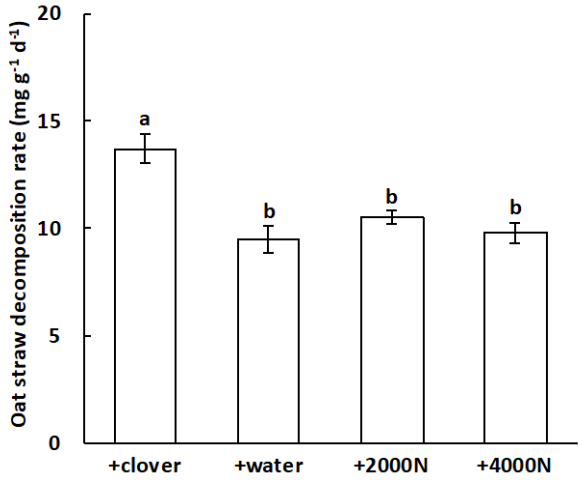

Figure 3.2. The effect of NH_4Cl amendment on mean decomposition rate of oat straw. + clover = oat straw with clover; + water = oat straw alone but with additional water; + 2 N = oat straw alone but with NH_4Cl at 2000 µg N as NH_4Cl g^{-1} dry weight of litter; + 4000 N = oat straw alone but with NH_4Cl solution at 4000 µg N as NH_4Cl g^{-1} dry weight of litter. Vertical lines are ± 1 SEM.

Experiment 3. C limits microbial activity in oat straw.

In experiment 3.1, which lasted only 1 week, there was no significant effect of glucose concentration on oat straw decomposition, but there was a significant effect of glucose

concentration on cellobiohydrolase activity. The concentration of 500 μg C as glucose g^{-1} dry litter produced the maximum cellobiohydrolase activity (Table 3.2, Figure 3.3). A previous experiment indicated that, after 4 weeks of decomposition, cellobiohydrolase activity in oat straw was correlated with oat straw decomposition rate (Figure 2.3b). Thus, in all subsequent experiments, I assumed the optimum glucose concentration for decomposition was 500 μg C as glucose g^{-1} dry litter.

Table 3.2. Results of one-way analysis of variance (ANOVA) for cellobiohydrolase activity in oat straw from Experiment 3.1.

Source of variation	df	SS	MS	*F*	*P*
Treatment	6	464706	77451	3.19	**0.0133**
Residuals	35	850788	24308		
Total	41	1315494			

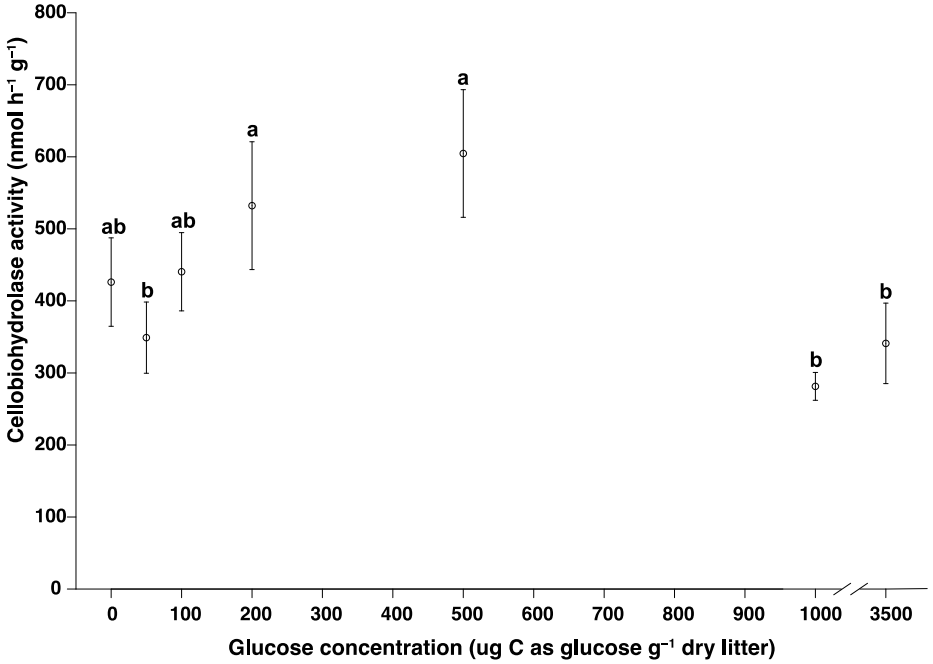

Figure 3.3. The effect of amendment of glucose at different concentrations on mean cellobiohydrolase activity in oat straw in Experiment 3.1. Vertical lines are ± 1 SEM.

Using data from experiments 3.2, 4, 5 and 6, I found a significant, positive linear relationship between oat straw decomposition and cellobiohydrolase activity (Figure 3.4). Thus, based on the cellobiohydrolase activities in Figure 3, in subsequent C addition experiments I have assumed that the optimum glucose concentration for decomposition was 500 μg C g^{-1} dry litter.

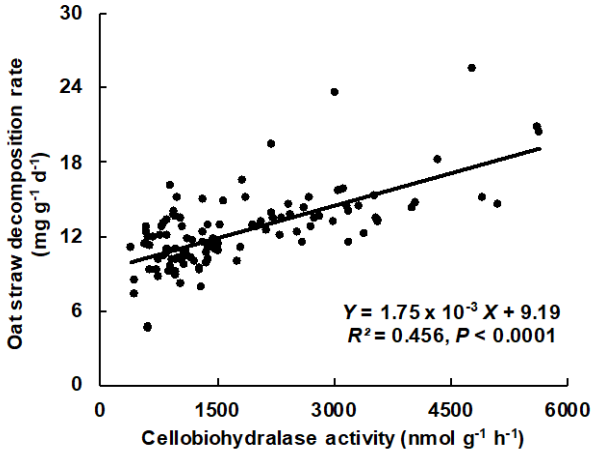

Figure 3.4. Relationships between oat straw decomposition rate and cellobiohydrolase activity in oat straw, data from Experiment 3.2, 4, 5 and 6.

Experiment 3.2. After 28 d of incubation, there was a significant effect of glucose treatment on oat straw decomposition, but not after 14 and 21 d of incubation (Table 3.3, Figure 3.5). For 21 d samples, a significant difference between the mixed oat straw treatment and the high glucose concentration treatment was found. For 28 d samples, clover significantly increased oat straw decomposition rate relative to single oat straw; the low glucose concentration treatment increased oat straw decomposition rate compared with the single oat straw treatment, but not significantly; the high glucose concentration treatment significantly reduced oat straw decomposition rate compared with the single oat straw treatment.

Table 3.3. Results of one-way analysis of variance (ANOVA) for oat straw decomposition at 14, 21 and 28 d of incubation in Experiment 3.2.

Incubation time	Source of variation	df	SS	MS	*F*	*P*
14 d	Treatment	3	4.67	1.56	0.218	0.883
	Residuals	27	192	7.13		
	Total	30	197			
21 d	Treatment	3	33.0	11.0	2.93	0.0511
	Residuals	28	105	3.76		
	Total	31	138			
28 d	Treatment	3	130	43.4	14.4	**<.0001**
	Residuals	28	84.3	3.01		
	Total	31	214			

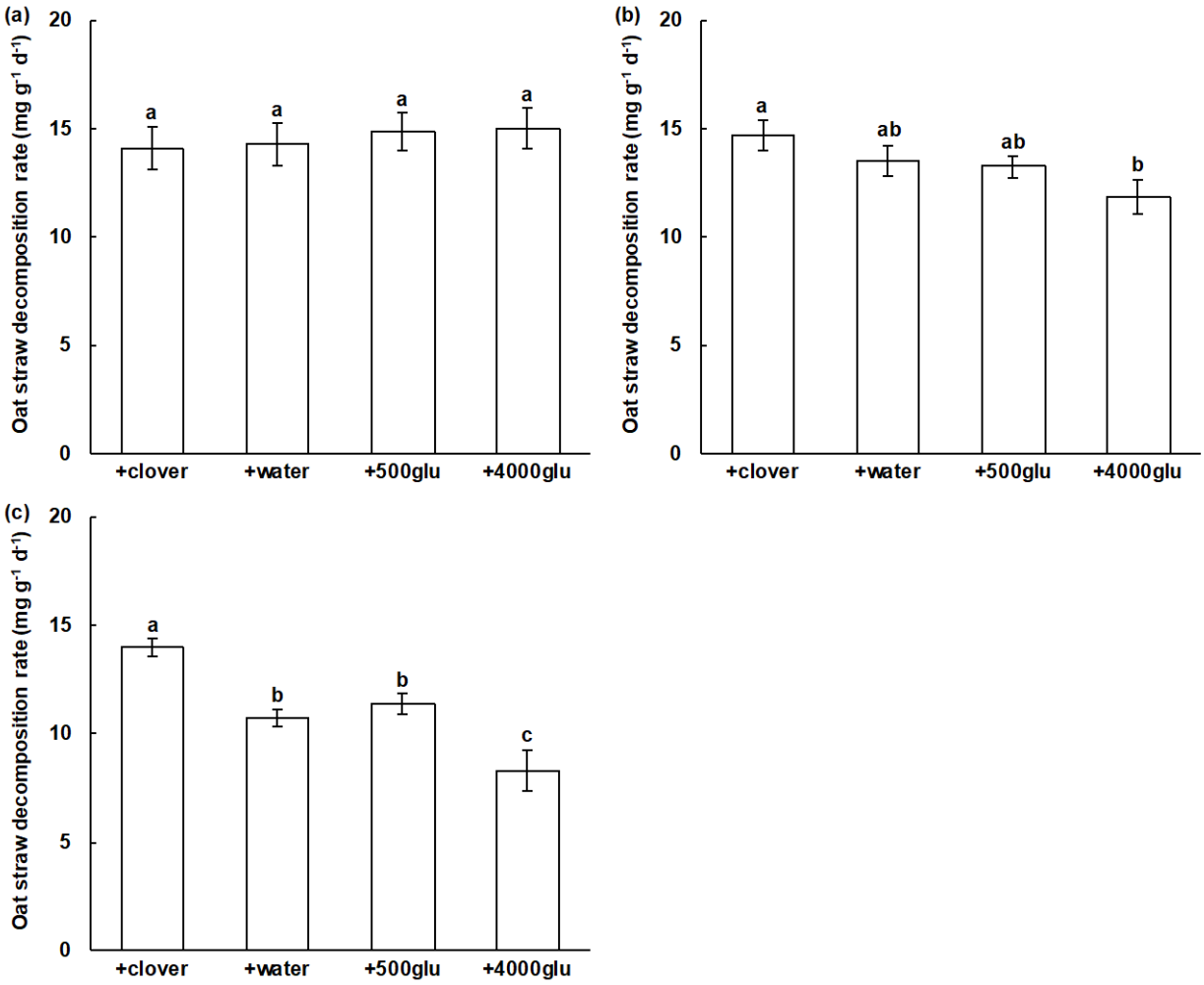

Figure 3.5. The effect of glucose amendment on mean decomposition rate of oat straw in Experiment 3.2 at 14 (a), 21 (b) and 28 (c) d of incubation. + clover = oat straw with clover litter; + water = oat straw with additional water supplied in an accompanying fiberglass pad; + 500 glu = oat straw with 500 µg C as glucose g-1 dry weight; + 4000 glu = oat straw with 4000 µg C as glucose g-1 dry weight. Vertical bars are ± 1 SEM.

For all three incubation times (14, 21 and 28 d), there was a significant effect of treatment on cellobiohydrolase activity (Table 3.4, Figure 3.6). Clover significantly increased cellobiohydrolase activity in oat straw relative to oat straw alone at 14, 21 and 28 d of incubation. The high glucose treatment significantly reduced cellobiohydrolase activity at 21 and 28 d of incubation.

Table 3.4. Results of one-way analysis of variance (ANOVA) for cellobiohydrolase activity in oat straw at 14, 21 and 28 d of incubation in experiment 3.2.

Incubation time	Source of variation	df	SS	MS	*F*	*F*
14 d	Treatment	3	6.61×10^{6}	2.20×10^{6}	48.7	**<.0001**
	Residuals	28	1.27×10^{6}	4.53×10^{4}		
	Total	31	7.88×10^{6}			
21 d	Treatment	3	4.17×10^{-6}	1.39×10^{-6}	45.4	**<.0001**
	Residuals	20	8.56×10^{-7}	3.06×10^{-8}		
	Total	31	5.02×10^{-6}			
28 d	Treatment	3	2.38	0.794	54.2	**<.0001**
	Residuals	28	0.389	0.0139		
	Total	31	2.77			

Notes: For 21 d samples, statistical analyses were performed on data that were reciprocally transformed; for 28 d samples, statistical analyses were performed on data that were log transformed.

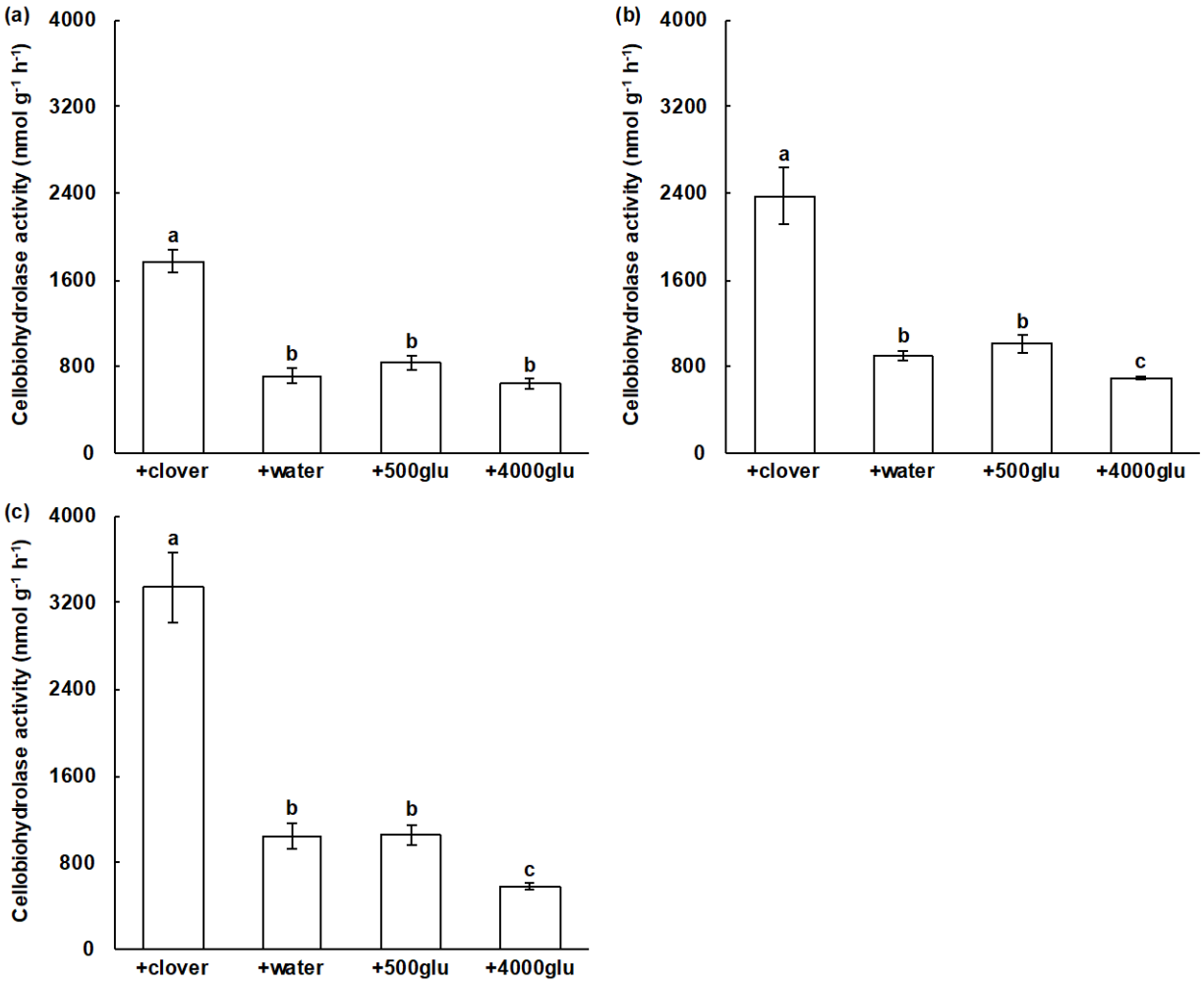

Figure 3.6. The effect of glucose amendment on mean cellobiohydrolase activity in oat straw in Experiment 3.2 at 14 (a), 21 (b) and 28 (c) of incubation. + clover = oat straw with clover; + water = oat straw alone but with additional water; + 500 glu = oat straw with 500 μg C as glucose g^{-1} dry weight of litter; + 4000 glu = oat straw with 4000 μg C as glucose g^{-1} dry weight of litter. Vertical lines are ± 1 SEM.

Experiment 4. C and N together limit microbial activity in oat straw.

After 28 d of incubation, there was a significant difference of mean oat straw decomposition rate among treatments (Table 3.5, Figure 3.7). Clover significantly increased oat straw decomposition relative to single oat straw. Compared with the single oat straw treatment, the glucose treatment significantly increased oat straw decomposition rate and was not

significantly different from the mixed oat straw treatment. Glucose and NH_4Cl treatment also increased oat straw decomposition rate compared with the single oat straw treatment but was not significantly different, indicating that C and N together are not the limiting resources for oat straw decomposition.

Table 3.5. Results of one-way analysis of variance (ANOVA) for oat straw decomposition.

Source of variation	df	SS	MS	*F*	*P*
Treatment	3	53.5	17.8	4.87	**0.0075**
Residuals	28	102	3.66		
Total	31	156			

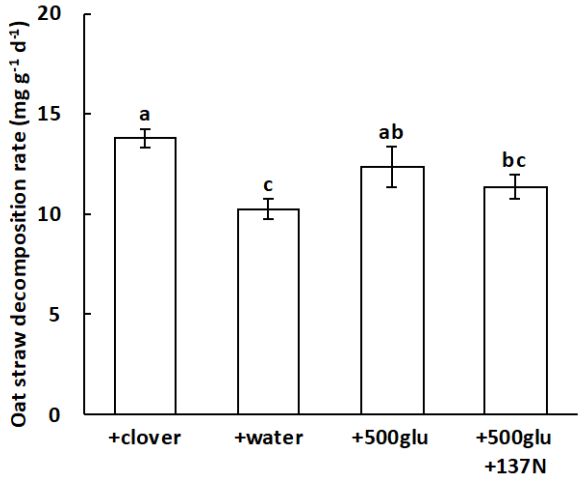

Figure 3.7. Mean decomposition rate of oat straw on the effect of glucose and NH_4Cl amendment. + clover = oat straw with clover; + water = oat straw alone but with additional water; + 500 glu = oat straw with 500 µg C as glucose g^{-1} dry weight of litter; + 500 glu + 137 N = oat straw with glucose (500 µg C as glucose g^{-1} dry weight of litter) and NH_4Cl (137 µg N as NH_4Cl g^{-1} dry weight of litter). Vertical lines are ± 1 SEM.

There was a significant difference in mean cellobiohydrolase activity among treatments (Table 3.6, Figure 3.8). Clover significantly increased oat straw decomposition relative single oat straw. Both glucose treatment and glucose and NH_4Cl together treatment had no significant effect on cellobiohydrolase activity compared with the single oat straw treatment.

Table 3.6. Results of one-way analysis of variance (ANOVA) for cellobiohydrolase activity in oat straw.

Source of variation	df	SS	MS	*F*	*P*
Treatment	3	1.67	0.556	40.9	**<.0001**
Residuals	28	0.381	0.0136		
Total	31	2.05			

Notes: Statistical analyses were performed on data that were log transformed.

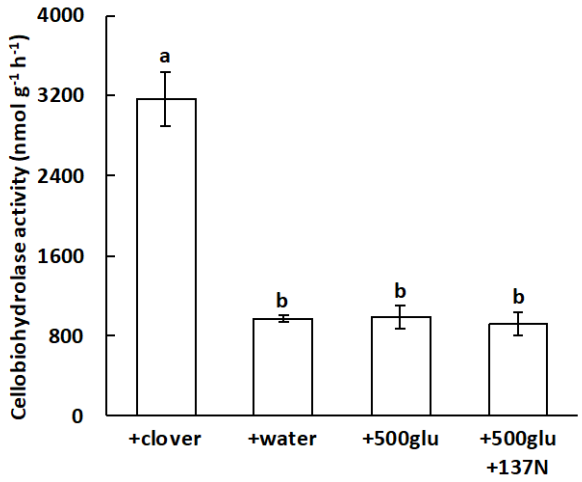

Figure 3.8. The effect of glucose and NH_4Cl amendments on mean cellobiohydrolase activity in oat straw. + clover = oat straw with clover; + water = oat straw alone but with additional water; + 500 glu = oat straw with 500 µg C as glucose g^{-1} dry weight of litter; + 500 glu + 137 N = oat straw with glucose (500 µg C as glucose g^{-1} dry weight of litter) and NH_4Cl (137 µg N as NH_4Cl g^{-1} dry weight of litter). Vertical lines are ± 1 SEM.

Experiment 5. C, N and other mineral nutrients together limit microbial activity in oat straw.

After 28 d of incubation, there was a significant effect of treatment on mean oat straw decomposition rate (Table 3.7, Figure 3.9). The rate of oat straw decomposition increased significantly when mixed with clover. The addition of glucose, NH_4Cl, and other mineral nutrients together also significantly increased oat straw decomposition rate, indicating that some

combination of C, N, and other mineral nutrients are limiting oat straw decomposition and that this combination is sufficient to account for the clover effect.

Table 3.7. Results of one-way analysis of variance (ANOVA) for mean oat straw decomposition rate.

Source of variation	df	SS	MS	*F*	*P*
Treatment	2	64.5	32.2	12.6	**0.0003**
Residuals	21	53.9	2.56		
Total	23	118			

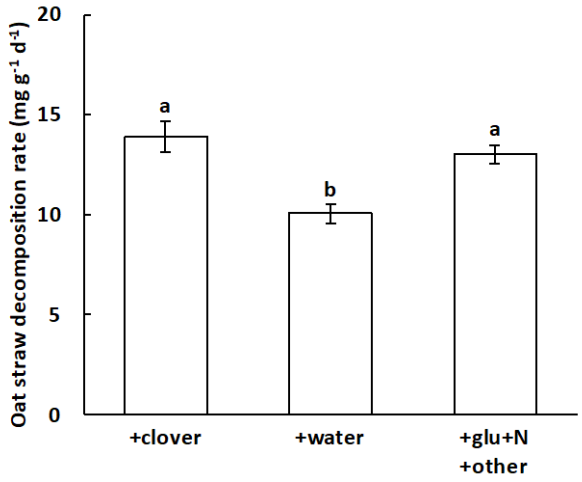

Figure 3.9. The effect of simultaneous amendment with glucose, NH_4Cl, and other mineral nutrients on mean cellobiohydrolase activity in oat straw. + clover = oat straw with clover; + water = oat straw alone but with additional water; + glu + N + others = oat straw with glucose (500 μg C as glucose g^{-1} dry weight of litter), NH_4Cl (137 μg N as NH_4Cl g^{-1} dry weight of litter), and all other mineral nutrients (for other mineral nutrient concentrations, see Methods) solution. Vertical lines are ± 1 SEM.

There was a significant effect of treatment on mean cellobiohydrolase activity in oat straw (Table 3.8, Figure 3.10). Clover significantly increased cellobiohydrolase activity in oat straw relative to oat straw alone. Glucose, NH_4Cl, and other mineral nutrients together also significantly increased cellobiohydrolase activity compared to oat straw alone, but it was significantly lower than in the mixed oat straw treatment.

Table 3.8. Results of one-way analysis of variance (ANOVA) for cellobiohydrolase activity in oat straw.

Source of variation	df	SS	MS	*F*	*P*
Treatment	2	0.801	0.401	50.6	**<.0001**
Residuals	21	0.166	0.0079		
Total	23	0.967			

Notes: Statistical analyses were performed on data that were log transformed.

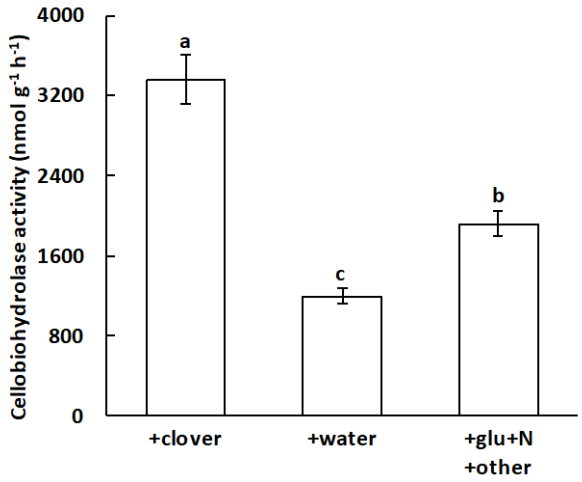

Figure 3.10. The effect of simultaneous amendment with glucose, NH_4Cl, and other mineral nutrients on mean cellobiohydrolase activity in oat straw. + clover = oat straw with clover; + water = oat straw alone but with additional water; + glu + N + others = oat straw with glucose (500 µg C as glucose g^{-1} dry weight of litter), NH_4Cl (137 µg N as NH_4Cl g^{-1} dry weight of litter), and all other mineral nutrients (for other mineral nutrient concentrations, see Methods) solution. Vertical lines are ± 1 SEM.

Experiment 6. Soil can supply limiting mineral nutrients to microbes to decomposing oat straw.

After 28 d of incubation, there was a significant effect of treatment on mean oat straw decomposition rate (Table 3.9, Figure 3.11). Clover significantly increased oat straw decomposition rate relative to oat straw alone. Simultaneous amendment with glucose, NH_4Cl, and soil also significantly increased oat straw decomposition rate compared to oat straw alone and the resultant decomposition rate was not significantly different from that of the mixed oat

straw treatment. This suggests that glucose, N, and soil together were also sufficient to account for the clover effect.

Table 3.9. Results of one-way analysis of variance (ANOVA) for oat straw decomposition.

Source of variation	df	SS	MS	*F*	*P*
Treatment	3	0.110	0.0368	3.55	**0.033**
Residuals	20	0.207	0.0104		
Total	23	0.318			

Notes: Statistical analyses were performed on data that were log transformed.

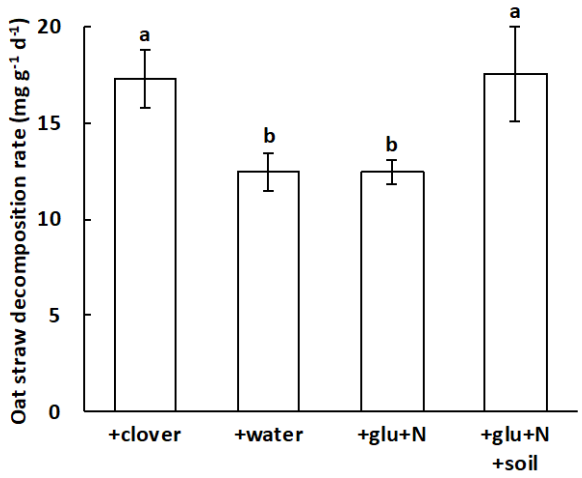

Figure 3.11. The effect of treatment on mean decomposition rate of oat straw. + clover = oat straw with clover; + water = oat straw alone but with additional water; + glu + N = oat straw with glucose (500 µg C as glucose g^{-1} dry weight of litter) and NH_4Cl (137 µg N as NH_4Cl g^{-1} dry weight of litter); + glu + N + soil = oat straw with glucose (500 µg C as glucose g^{-1} dry weight of litter), and NH_4Cl (137 µg N as NH_4Cl g^{-1} dry weight of litter) solution on sterilized soil. Vertical lines are ± 1 SEM.

There was a significant effect of treatment on mean cellobiohydrolase activity in oat straw (Table 3.10, Figure 3.12). Clover significantly increased cellobiohydrolase activity in oat straw relative to oat straw alone. Glucose and NH_4Cl did not significantly influence cellobiohydrolase activity. Simultaneous amendment with glucose, NH_4Cl, and soil significantly

increased cellobiohydrolase activity, but this activity was significantly lower than in the mixed oat straw treatment.

Table 3.10. Results of one-way analysis of variance (ANOVA) for cellobiohydrolase activity in oat straw.

Source of variation	df	SS	MS	*F*	*P*
Treatment	3	0.945	0.315	20.1	**<.0001**
Residuals	19	0.298	0.0157		
Total	22	1.24			

Notes: Statistical analyses were performed on data that were log transformed.

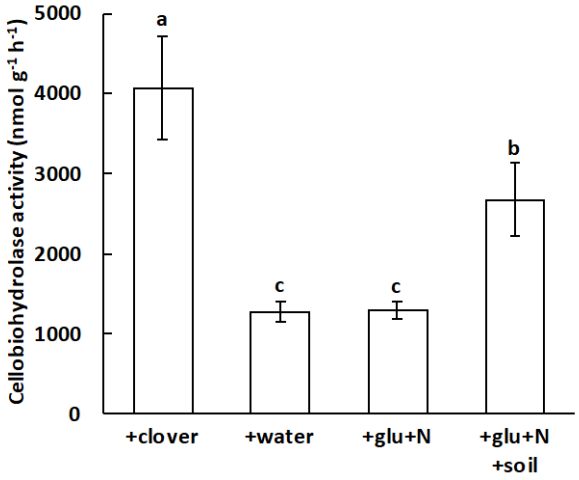

Figure 3.12. The effect of treatment on mean cellobiohydrolase activity in oat straw. + clover = oat straw with clover; + water = oat straw alone but with additional water; + glu + N = oat straw with glucose (500 µg C as glucose g^{-1} dry weight of litter) and NH_4Cl (137 µg N as NH_4Cl g^{-1} dry weight of litter); + glu + N + soil = oat straw with glucose (500 µg C as glucose g^{-1} dry weight of litter) and NH_4Cl (137 µg N as NH_4Cl g^{-1} dry weight of litter) solution on sterilized soil. Vertical lines are ± 1 SEM.

DISCUSSION

We previously showed (Chapter 2) that oat straw decomposition was stimulated by the presence of clover litter, and that this stimulation was associated with the significantly increased microbial activity. The purpose of the research reported in this chapter was to determine the

mechanisms by which clover litter stimulates the activity of microbes decomposing oat straw, thus stimulating oat straw decomposition. We tested several hypotheses having to do with the supply of limiting resources from clover litter to oat straw microbes including water, N, reduced C, other minerals, or various combinations of these.

Moisture can be a limiting factor for microbial activity during litter decomposition (Wardle et al. 2003, Hättenschwiler et al. 2005). Therefore, I first tested the hypothesis that the supply of water by clover litter to oat straw stimulates oat straw decomposition. We reckoned that clover litter is most capable of supplying water when it is saturated with water. However, if clover litter were saturated under natural circumstances, the adjacent oat straw would also be saturated. Therefore, in our experiment I used saturated oat straw and saturated clover litter, and simulated the water availability from clover litter using inert fiberglass containing water. Moreover, water loss was minimized by the humidified incubation enclosure. Under those conditions, our results were not consistent with the hypothesis that the transfer of water from clover litter to oat straw increased the decomposition rate of oat straw. In fact, I can envision only one circumstance when water transfer could be responsible for positive, non-additive decomposition, which is when water is lost only from the oat straw, and is replaced only by water from the clover litter. In that case the time during which the water potential of oat straw remains favorable for decomposition could be increased by the clover litter. If, on the other hand, there were hydraulic connectivity between soil and either litter type, the water potential decline of oat straw would be the same with or without clover litter because it would be governed by the rate of soil drying. In short, while it is possible for water transfer from one litter type to the other to be responsible for positive, non-additive decomposition, this does not seem likely under the

conditions when I have observed positive, non-additive decomposition, namely when water loss is minimized in a high humidity enclosure.

N frequently limits productivity in forested ecosystems. Some have proposed that the transfer of N from one litter type to another can cause positive, non-additive decomposition and, indeed, this has been shown (Liu et al. 2007). Because the N concentration in our oat straw is significantly lower than in our clover litter, I hypothesized that clover litter enhanced oat straw decomposition by supplying N to oat straw microbes. However, the addition of NH_4Cl at either 2 mg N g^{-1} dry weight of litter nor 4 mg N g^{-1} dry weight of litter had no significant impact on oat straw decomposition, suggesting that microbial activity and oat straw decomposition were not limited by N. Other researchers have also shown that in some circumstances N transfer alone is not responsible for the positive, non-additive effect so frequently observed (Smith and Bradford 2003, Chen et al. 2013). In our case, it is possible that NH_4Cl was not readily usable by the saprotrophic microbes and thus was not a good model N source. Perhaps organic N sources such as protein or amino acid would have acted differently.

Priming is the case in which the decomposition of soil organic matter or litter is stimulated by the addition of a readily available energy source in the form of soluble reduced C (Hamer and Marschner 2005). In a preliminary experiment, I supplied various concentrations of glucose to oat straw in order to ascertain whether its decomposition was limited by C availability. We had previously found that oat straw decomposition and oat straw cellobiohydrolase activity were correlated (Chapter 2). In the preliminary experiment, I found that glucose concentration influenced cellobiohydrolase activity, increasing it from 50 to 500 μg C as glucose g^{-1} dry litter, but decreasing it at 1000 and 3500 μg C g^{-1} dry litter. We assumed,

therefore, that 500 μg C as glucose g^{-1} dry litter would stimulate oat straw decomposition and 4000 μg C as glucose g^{-1} dry litter would retard oat straw decomposition.

In our main glucose experiment, I therefore chose as contrasting experimental treatments 500 and 4000 μg C as glucose g^{-1} dry litter. We did, indeed, find that 4000 μg C as glucose g^{-1} dry litter retarded oat straw decomposition. Negative effects of glucose additions on decomposition has been reported previously (Boberg et al. 2008, Chigineva et al. 2009). This negative impact of glucose on litter decomposition may be due to the labile glucose being preferentially metabolized over the more recalcitrant C sources in litter. That was supported by the strong negative effect of high concentrations of glucose on cellobiohydrolase activity in our study. We did find that 500 μg C as glucose g^{-1} dry litter increased oat straw decomposition, but not significantly. Thus, it does not appear that glucose alone, at any concentration could stimulate oat straw decomposition in a manner comparable to clover litter.

In the glucose and NH_4Cl amendment experiment, the results show that the combination of glucose and NH_4Cl actually retarded oat straw decomposition relative to glucose alone, although not significantly, and that the combination did not stimulate oat straw decomposition to the extent that clover litter did. We conclude, therefore, that if clover litter enhances oat straw decomposition by supplying limiting resources, they are not simply C and N.

However, when I added to oat straw a combination of glucose, NH_4Cl, and all other mineral nutrients considered to be essential for plant growth, their combined effect on oat straw decomposition was indistinguishable from the effect of clover litter. This suggests that in our system, clover supplies a wide range of nutrients that may limit the activity of saprotrophic microbes responsible for decomposing oat straw. While soil is likely to be deficient in both C and N for microbial growth, it generally contains phosphate, potassium, and a wide range of

mineral nutrients. Thus, when I added glucose, NH_4Cl, and soil to oat straw, its decomposition was again stimulated to the same extent as when it was amended with clover litter. In both the glucose, NH_4Cl, and all other mineral nutrients experiment and the glucose, NH_4Cl, and soil experiment, cellobiohydrolase activity was stimulated by the amendments, but not to the same extent as clover litter. Thus, both types of amendments simulated the effect of clover litter on short term decomposition of oat straw, but not on short term enzyme activity. The reason for this remains obscure, but it suggests that there is yet something I do not know about the mechanism by which clover litter influences oat straw decomposition.

Positive, non-additive decomposition is certainly a complex phenomenon. Undoubtedly there are many mechanisms by which it occurs, depending on the circumstances. In the absence of moisture limitations, the amendment of oat straw with C (glucose) alone, N (NH_4Cl) alone, and the combination of C and N were insufficient to simulate amendment with clover litter. However, the combination of C, N, and other mineral elements, either supplied by nutrient salts or by soil, was sufficient to simulate the effect of clover litter on oat straw decomposition. In our simple agricultural model consisting of clover litter and oat straw, clover litter apparently supplies oat straw microbes with multiple resources. In nature, litter frequently decomposes in the presence of other litter types and soil. Thus, positive non-additive decomposition may be due to resources being supplied to microbes decomposing the litter from a combination of other litter types and soil. Thus, whether positive, non-additive decomposition occurs is not simply a function of the two litter types involved, but also the surrounding matrix (frequently soil).

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