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Soils are a globally significant carbon (C) pool and have the potential to respond to elevated CO_2 and environmental changes through positive feedback cycles that enhance the turnover of soil organic matter (SOM). Understanding the mechanisms governing the turnover of SOM is particularly important for modeling the fate of C in soils under predicted environmental changes. The change in turnover of SOM following additions of labile C through natural root and litter deposition as well as from human activities such as fertilizer application is known as priming, and the mechanisms governing this process are poorly understood but may be important components determining soil C balance. Many studies have utilized experimental additions of labile C and variously interpreted the primed C to derive from the activation and turnover of microbial biomass or from the increased decomposition of non-microbial SOM. The objective of this study was to evaluate changes in the activities of SOM-degrading enzymes as a potential mechanism for observed priming effects and to determine whether the priming response was related to the availability of "primable" C from root and litter inputs to soil.

^{13}C-labeled glucose was injected in the field into surface soils of an old-growth coniferous forest on the western side of the central Oregon Cascade Range. The fate of these small glucose additions (100 μg C g^{-1} dry soil) was traced into soil C pools and captured as respired CO_2 over the course of a 22-day experiment. The forest soils considered in this study have undergone ten years of selective exclusion of root and/or litter inputs as part of a larger experiment known as the Detrital Input and Removal

Treatments (DIRT). Plots without roots showed negative priming responses to glucose additions with reduced turnover of SOM due to a preferential switch from SOM to glucose as a C source and through an apparent generalized suppression of microbial activity. Positive priming was observed in soils receiving regular C inputs from roots and litter. Both positive and negative priming were observed in plots with litter inputs excluded depending upon which method of priming quantification was used. In soils with C-input limitations (i.e., No Roots, No Litter, No Inputs), the magnitude and direction of priming was negatively related to background respiration rates with highly active soils in these plots showing the strongest negative priming effects. Control soils showed no relationship between background respiration rates and priming effects.

The potential activities of β-glucosidase, phenol oxidase, and peroxidase showed no consistent relationship to glucose additions or priming effects and suggest that the production of enzymes in response to labile C inputs may not be the controlling mechanism for priming in these soils. The amount of primed C mineralized throughout the course of the experiment is consistent with earlier hypotheses that the activation and increased turnover of microbial biomass C is the primary source of primed C.

Modeling the turnover of glucose-derived C in soil revealed at least two pools of glucose-C with mean turnover times of 1 d and 30 d. The initial respiratory response of soils to glucose additions was dependent upon the rapid turnover of an easily degradable pool whereas $^{13}CO_2$ efflux after four days was tied more directly to the turnover of a slowly degrading pool. A significant portion of glucose-C remained in soils throughout the course of the experiment in a non-extractable pool comparable in size to the amount of glucose-C taken up into microbial cells.

The results of this study contribute new and challenging problems for mechanistic interpretation of SOM priming. The lack of a discernible connection between enzyme activities and SOM priming suggests that observed priming was not related to accelerated turnover of stable SOM but it also seems unlikely that microbes in this C-limited system would maintain large intracellular reserves of C. The relationship between background respiration rates and negative priming as well as the generalized suppression of microbial activity following glucose additions are novel observations and defy common expectations of glucose effects on microbial activity.

Carbon Cycling and Priming of Soil Organic Matter Decomposition in a Forest Soil Following Glucose Additions

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

David D. Diaz, Author

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Chapter 1:
General Introduction

David D. Diaz

Soils and Global Change

Soils are a vital resource for life on the planet. With the ongoing pressures of population growth, climate change, and decreases in biodiversity, the conservation of soil as a critical resource for sustaining life on the planet is fundamental to addressing the imperatives introduced by global change. For much of its early history, soil was appreciated primarily as a medium for plant growth and therefore a critical component to the sustainability of human and animal populations. More recent research documenting the effects of human activities on global climate has revealed the importance of soil in changes to the global carbon (C) cycle and therefore as an important component of climate change.

Globally, soils comprise the largest biologically active terrestrial pool of C. To one meter depth, soil C is estimated at 1500-2000 Pg globally, making it about four times the size of the biotic pool (e.g., plants and animals) and three times the size of the atmospheric pool (Janzen, 2004). Fluxes from the soil organic C (SOC) pool contribute 60 Pg of C to the atmosphere every year, which is approximately balanced by terrestrial C uptake (Janzen, 2004). By virtue of the size and close balance of fluxes of the soil C pool, even small changes in the soil C flux component in the global C cycle could contribute significant amounts of C to the atmosphere. Recognition of the importance of soil C in the global C cycle has led some to call for the integration of soil management into global climate change mitigation strategies (Lal, 2004).

Potential for Positive Feedbacks

The balance between the net uptake of atmospheric C by plants, or net primary production (NPP) and the turnover of SOM and consequent efflux of carbon dioxide (CO_2) from soils is pivotal to the evaluation of whether positive or negative feedbacks may occur in the terrestrial carbon cycle (Kirschbaum, 2000). Many studies utilizing elevated atmospheric CO_2 concentrations have shown both enhanced NPP and simultaneous increases in soil CO_2 efflux (Cheng, 1999). Feedback loops based on CO_2 fertilization of plants have been traced

to increases in root exudation which in turn stimulate microbial turnover of SOM resulting in accelerated cycling of soil nutrients and enhanced soil CO_2 efflux (Cheng, 1999). These demonstrated increases in inputs and outputs of soil C reveal the need for a more thorough mechanistic understanding of what processes govern the turnover of soil C, particularly in relation to demonstrated increases in root turnover and root exudation (collectively referred to as rhizodeposition) following elevated atmospheric CO_2 concentrations.

Carbon Inputs and Transformations in Soil

In order to evaluate the potential for soils to contribute to potential feedbacks in the global C cycle, it is necessary to consider the mechanisms governing the turnover of soil C. C inputs to soil include the above-ground litterfall from plant sources as well as the below-ground inputs of root turnover and root exudation. Once in the soil, organic C is decomposed and respired through microbial activity and returned to the atmosphere as CO_2, taken up into microbial biomass, excreted in the form of microbial metabolites and products, or remains in the soil as soil organic matter (SOM) (Post et al., 1990). At the most basic level, creating general characteristics of the pools and fluxes of C within soils is complicated by the diversity of microbial communities, the molecular complexity of SOM, and the inherent environmental heterogeneity of soil ecosystems.

Rates of initial decomposition of plant litter have been shown to correspond to the availability of N and the presence of complex plant compounds such as lignin (Melillo et al., 1982). In the old-growth forests of the Oregon Cascade Range, litter decomposition provides a significant input of C to soils, measuring up to approximately 150g C m^{-2} $year^{-1}$ (Sulzman et al., 2005). Experimental manipulations using litter additions have also resulted in increased respiratory activity above and beyond expected decomposition rates of the fresh litter suggesting an increased turnover of SOM, suggesting that there may be an interactive feedback between C inputs to soils and the turnover of preexisting SOM (Sulzman et al., 2005).

Below-ground C inputs into soil include root death and turnover and the exudation of a variety of low-molecular-weight organic compounds including organic acids, sugars, and

amino acids from plant roots (Jones et al., 2004). Quantification of the exact amounts of rhizodeposition have been problematic, but an average of approximately 2-4% of photosynthetically fixed C is believed to enter the soil through root deposits (Jones et al., 2004). The transfer of recently fixed photosynthate to plant roots and into the soil exerts a strong control over soil metabolic activity and may drive upwards of 50% of soil respiration (Högberg et al., 2001; Högberg et al., 2006). The connection between plant fixation and soil respiration is a fairly quick and is believed to operate on a lag time of 2-4 days (Högberg et al., 2001; Högberg et al., 2008). Glucose concentrations in an agricultural soil where corn is grown have been estimated to range as low as 0.02 μg glucose-C g^{-1} dry soil (1-10 μM) following dilution due to rain events to as high as 205 μg glucose-C g^{-1} dry soil (1,000-10,000 μM) upon root death and lysis of root cells (Boddy et al., 2007).

Following initial decomposition of plant litter and rhizodeposits, the fate of SOC is not well understood. The idea that organic matter is preserved by biochemical "recalcitrance,"—an inherent resistance to microbial degradation—has been a prevailing paradigm for the creation of stable SOM for decades. The importance of recalcitrance is now being drawn into question based on recent studies utilizing technologies which were not available during the development of these earlier theories, such as nuclear magnetic resonance (NMR) and pyrolysis techniques which enable the molecular characterization of SOM throughout various periods of decomposition (Marschner et al., 2008). Studies of the relative amounts of different chemical classes of compounds in SOM show that the presence of compounds which are thermodynamically "difficult" to degrade such as aromatics and phenolics are not selectively preserved in SOM and that compounds such as alkyl-C and O-alkyl-C generally predominate the makeup of stable SOM (Marschner et al., 2008). The fate of stable SOM, which has turnover times on the order of centuries to millennia has been drawn into the debate over feedbacks of soil C to climate change and much work remains to be done to trace the fate of C in this stable SOM pool following the cascading effects of increased atmospheric CO_2 concentrations. Specifically, it is not known whether potential feedbacks under elevated CO_2 result in the mineralization of stable SOM or instead from pools of more short-lived SOC, such as microbial biomass.

Current models of soil C commonly evaluate the turnover of SOC based on soil and atmospheric climate variables, pedologic features such as the amount and/or type of clay present in soil, as well as the inherent "recalcitrance" or resistance to decomposition of C inputs and pools (Powlson et al., 1996). Such SOM and SOC models may prove useful in predicting responses of soils to changes in climate and C inputs, but the mechanisms governing soil C cycling have yet to be completely integrated. One particular aspect of soil C cycling that has drawn considerable attention and resisted simple explanation since its discovery is the priming effect of C additions on the turnover of native SOM.

Priming of SOM

Priming effects have been defined as "strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil" (Kuzyakov et al., 2000). As early as 1926, experimental additions of organic substrates were interpreted to result in increased mineralization of SOM (Löhnis, 1926). Direct demonstration of priming occurred after the introduction of stable isotope methods and was performed by Broadbent and Norman using ^{13}C-labeled plant material (Broadbent et al., 1946). Early experiments investigating priming included use of a variety of both ^{13}C- and ^{14}C-labeled plant materials and glucose in both single and repeated additions, revealing numerous positive and negative priming responses following substrate additions, but were generally not presented in such a way that the statistical significance of the priming action could be judged (Jenkinson, 1966). Experimentally, the priming effect is usually determined by partitioning soil CO_2 efflux into CO_2 derived from an isotopically-labeled substrate and CO_2 derived from the oxidation of endogenous SOM (i.e., all pools of SOC). Changes in the amount of SOM-derived CO_2 compared to a non-amended control soil provide a quantitative estimate of the change in turnover of SOM. Increases in the turnover of SOM are referred to as positive priming; decreases are referred to as negative priming. Apart from various labeled plant materials, glucose has been a commonly used as an organic substrate in priming experiments, likely because it is a C compound widely metabolized by soil microbes as well as the fact that

glucose is typically the largest measured component of root exudates (Ketchie et al., 1981; Hütsch et al., 2002; Derrien et al., 2004; Hill et al., 2008).

Mechanisms Governing SOM Priming

The evidence for priming holds a paradoxical relationship to the traditional maxim that the metabolic activity of soil microbes is limited by the availability (including the "recalcitrance") of soil C. Given this assumption, it appears contradictory that additions of exogenous C would stimulate the turnover of endogenous (i.e., preexisting) soil C for which the microbes are assumed to have utilized to their fullest ability based on C limitation. Recent research on the assumption of microbial C limitation has suggested that though this assumption may hold in bulk soil, in close proximity to roots C availability does not appear to be the most limiting factor (Cheng et al., 1996). Because most experimental manipulations considering the priming effect are methodologically incapable of separating rhizosphere soil from bulk soil, and due to the fact that priming is of interest not only in relation to rhizodeposition but in relation to organic amendments to soil more generally, C limitation in bulk soil metabolic activity remains a common assumption. Investigating the paradoxical increase of SOM turnover lies primarily in determining the source(s) of CO_2 derived from primed SOM.

A variety of terminology has been used in the literature concerning "real" or "apparent" priming effects that is inconsistently applied across studies. For the sake of clarity in this study, priming effects will not be differentiated according to the somewhat arbitrary real/apparent distinction, but rather by considering the source for the observed primed CO_2. Though our working definition of priming is restricted to turnover of SOM, there remains the potential for errors in priming estimates due to exchanges of labeled C with forms of unlabeled soil inorganic carbon (SIC). The derivation of primed CO_2 from SIC is driven by experimental artifacts from the exchange of isotopically-labeled CO_2 with unlabeled C in inorganic forms such as $CaCO_3$ (Jenkinson, 1966). When considering this as a source of error, it should be noted that even in a calcareous soil where this process was observed, the amount of labeled CO_2 released by acid-treatment of soil was small compared

to total observed priming effect (Jenkinson, 1966). A variety of other experimental artifacts may contribute to errors in evaluating priming, but they generally correspond to treatments in which both C and N additions are made or where organic substrates on not uniformly labeled with ^{13}C or ^{14}C (Kuzyakov et al., 2000).

Looking more directly at the turnover of SOM, the potential sources of primed CO_2 are commonly divided into two pools

1. Non-microbial SOM (NM-SOM), which includes stable SOM, any labile SOC/SOM, such as dissolved organic carbon (DOC), and microbial metabolites outside of the cell;
2. Microbial biomass carbon (MBC).

Non-Microbial SOM as a Source of Primed Carbon

The decomposition of SOM is performed by a variety of exocellular enzymes produced by soil microorganisms and by the direct microbial uptake and metabolism of low-molecular-weight organic molecules. Thus, any observed increases in the turnover of NM-SOM are generally expected to follow increases in the decomposition of SOM by soil microbes or more specifically through simultaneous changes in the amount or activities of SOM-degrading enzymes (Weintraub et al., 2007). Several priming studies have looked directly at enzyme activities in relation to organic amendments, but the results have been inconsistent or contradictory. The results from these types of studies are summarized in Table 1.1. It should be noted that the activities reported in these studies correspond to potential enzyme activities determined by methods that saturate a sample with a fluorometric or colorimetric substrate and that activities presented in these studies are analogous to the number of active enzymes rather than a measure of a specific enzyme activity *in situ.* Thus, increases in potential enzyme activities would correspond to increased production of enzymes by soil microbes in response to substrate additions. One potential means for soil enzymes to be responsible for increased priming of soil organic matter without simultaneously increasing in number (i.e., no change in potential activity) is through the mobilization or destabilization of formerly stable SOM due to increased exudation of organic

acids by plants or soil microorganisms, though this hypothesis has yet to be experimentally measured in relation to priming (Kuzyakov et al., 2000).

Microbial Biomass as a Source of Primed Carbon

The second pool generally evaluated as the potential source for primed CO_2 is the microbial biomass. CO_2 can be generated from biomass either from an increase in the death and turnover of dead microbial cells or from the metabolism of intracellular C reserves. At glucose concentrations well below those commonly utilized in priming experiments, between 10-100 μM (~0.21-2.05 μg glucose-C g^{-1} dry soil), glucose uptake and metabolism operates according to at least two transport systems, one that results in rapid mineralization at low concentrations and a second low-affinity transport system that produces a lag between glucose uptake and mineralization revealing an intracellular pool C, which may be rapidly-replenished C (Hill et al., 2008). Ultimately however, the total amount of C available in the microbial biomass pool is limited and any priming effects that produce more CO_2 from SOM than is present in MBC must be derived in part from the increased turnover of NM-SOM. One of the major difficulties introduced in evaluating the role of MBC in SOM priming is the common experimental addition of organic substrates in concentrations that not only far outstrip natural rhizodeposition rates, but also that contain an order of magnitude or more C in the substrate than is contained in microbial biomass. These large substrate additions commonly produce biomass growth effects which may complicate interpretation of priming mechanisms that have been observed to occur even with substrate additions as low as 11.3 μg substrate-C g^{-1} dry soil, or approximately 3-6% of MBC (De Nobili et al., 2001). In the studies that have utilized enzyme assays in relation to priming at low-dose levels, only one has been completed in the field and the researchers found little relationship between enzyme activities and the cessation of root exudation via girdling (Weintraub et al., 2007). To evaluate the importance of priming that may be expected to occur in the natural world, it is necessary to determine whether the enzyme effects observed in other studies are due to the enhanced growth of microbial biomass and subsequent demand for more C and nutrients

because of large C additions or whether the enzyme response is something that occurs without substantial growth of microbial biomass.

Many of the priming studies conducted following the development methods for the quantification of microbial biomass demonstrated significant growth affects following substrate additions. In an innovative experimental design, Dalenberg and Jager (1981) created ^{14}C-labeled SOM (including MBC) and then applied a glucose addition to evaluate potential sources for the priming of SOM by monitoring the evolution of $^{14}CO_2$. The authors found an increase in $^{14}CO_2$ evolution of 3-7% above background respiration shortly after additions of 570 and 870 μg glucose-C g^{-1} dry soil (Dalenberg et al., 1981). The small amount of priming was interpreted to be from the enhanced turnover of microbial biomass, which the authors further pursued by culturing microbes from the soil using ^{14}C-labeled glucose, repeatedly fumigating them with chloroform to determine the amount of $^{14}CO_2$, adding soil infusions to introduce "not or only very weakly labelled" biomass, and then adding unlabeled glucose (Dalenberg et al., 1981). Following up on this study, Dalenberg and Jager (1989) pursued microbial biomass as the source of primed CO_2 more directly. By utilizing culture-grown microbes in solution and then administering additions of monosodium glutamate (msg), the authors found a linear correlation between the amount of microbial biomass in suspension and the amount of primed CO_2 (Dalenberg et al., 1989). Comparing the priming effects from additions of msg (12,800 μg C) in a ^{14}C-labeled biomass (~18,000 μg of microbial biomass) grown in perlite to additions of msg (920 μg C g^{-1} dry soil), aspartic acid (1030 μg C g^{-1}dry soil), and glucose (1140 μg C g^{-1} dry soil) in ^{14}C-labeled soil, the authors demonstrated that the priming effect in soil incubations was a fraction of that found from the priming effect due to biomass alone, suggesting that microbial biomass carbon could explain most, if not all, of the primed CO_2 (Dalenberg et al., 1989). Wu et al. (1993) conducted an experiment using both labeled glucose and ryegrass at high and low concentrations of 500 and 5000 μg C g^{-1} dry soil for both substrates. They observed priming by glucose only in the larger addition whereas priming occurred in both additions of ryegrass. They concluded that the amount of primed CO_2 from glucose was roughly equivalent to the measured turnover of microbial biomass, whereas the priming effect was

too large in the ryegrass additions to be explained by microbial biomass alone (Wu et al., 1993).

Microbial Competition Theory for Priming

Based on the understanding that soil microbes show different growth patterns and substrate specializations, Fontaine et al. (2003) put forward a hypothesis considering both potential CO_2 sources together. They suggest that the priming effect may derive from competition among soil microbes with two different growth and substrate strategies: **r-selected** vs. **K-selected organisms**. r-selected organisms specialize in the degradation of simple C substrates, are able to respond rapidly to the labile C inputs with rapid growth of biomass, and may produce exocellular enzymes that may only be weakly effective degraders of SOM; whereas K-selected organisms generally prefer more complex C substrates, produce more effective SOM-degrading exocellular enzymes, dominate soil activity during periods of low nutrient availability, and can metabolize simple C substrates slowly and with sluggish reproductive responses. The authors suggest that following additions of labile substrates, any conditions enabling growth of K-selected microorganisms such as N limitation or absence of competition will result in increased mineralization of SOM either through the production of exocellular enzymes that degrade SOM or through the eventual turnover of biomass during returns back to steady-state levels (Fontaine et al., 2003). According to Fontaine et al. (2003), no evidence of enzyme production in relation to substrate availability had yet been demonstrated in soils and therefore it can be assumed that enzymatic effects in the soil environment must be tied directly to biomass growth. This assumption may conflict with several of the studies presented earlier, although the issue of separating growth of microbial biomass from enzyme production complicates this issue considerably. The study which may most directly conflict with this assumption is that of Ladd and Paul (1973) who observed changes in casein-hydrolyzing and benzoyl arginine amide-hydrolyzing enzymes with glucose additions of 3000 μg glucose-C g^{-1} dry soil that did not coincide with increased viable bacterial populations. Assuming enzyme production (and therefore potential activities) is directly tied to growth of microbial biomass, the only means of producing

increases in efficient SOM-degrading enzymes is through the growth of K-selected microorganisms (Fontaine et al., 2003). At naturally occurring levels of root exudation, this assumption has been upheld by Weintraub et al. (2007) who found only one significant relationship out of nine enzymes considered between cessation of root exudation resulting from tree girdling and potential exocellular enzyme activities. The distinction between biomass growth and enzyme production may become particularly relevant as a recent review of studies on the effects of elevated CO_2 for fine roots and soil organisms found that microbial biomass on average increased with elevated CO_2 but that variation between studies and ecosystems was very high: beneath graminoid species, biomass averages increased +17% ± 86 SD; for herbaceous species +29% ± 29 SD; for woody species +19% ± 45 SD (Zak et al., 2000).

Comparisons of substrate-induced respiratory (SIR) responses believed to derive from r-selected and K-selected growth have been performed in few studies to this point in relation to priming but do suggest that rapid transitions may occur between r and K dominance. In the context of SIR, r-selected species comprise those organisms that respond immediately to substrate additions (usually glucose) with oxidation and growth and those who do not respond with growth are classified as K-organisms (Stenstrom et al., 1998). Thus, the growth of microorganisms is considered to correspond to the production of enzymes from each respective group (Stenstrom et al., 1998). Strenstrom et al. (2001) later demonstrated that microbial biomass may transition back and forth between r and K dominance depending upon the amount of glucose added, and that even with glucose additions as low as 110-335 μg glucose-C g^{-1} dry soil, after four days the soil microbial community appears to be overwhelmingly dominated by the growth of r-selected microorganisms. The only experiment to date which has directly examined priming mechanisms in relation to r versus K competition used low (48.7 μg glucose-C g^{-1} dry soil) and high (4870 μg glucose-C g^{-1} dry soil) substrate additions crossed with N additions to evaluate the role of C- and N-limitation in priming (Blagodatskaya et al., 2007). The researchers found that the small glucose additions produced positive priming effects whereas large glucose additions resulted in negative priming effects. At both low and high substrate additions, adding N produced more

negative priming effects compared to additions of C substrates alone (Blagodatskaya et al., 2007). Blagodatskaya et al. (2007) found changes in the dominance of r versus K SIR responses over the course of the experiment and that CO_2 efflux following substrate additions amounted to a maximum of about 30% of the carbon determined to reside in microbial biomass via SIR methods. The authors then concluded that microbial biomass carbon was likely the source of primed C in their experiments and that accelerated microbial metabolism due to competition between r and K organisms was responsible for the observed priming effect.

Responses of Diverse Microbial Communities to Substrate Additions

The microbial community in soils is very diverse, and as demonstrated in the r versus K-type experiments, it would be reasonable to expect that different members of the microbial community would respond more strongly to others following the inputs of new C substrates. Researchers have examined the responses of microbial communities to rhizodeposition and their role in the priming action both directly through molecular characterization and indirectly through manipulation of microbial habitat prior to priming assessments. Göttlicher et al. (2006) performed a girdling experiment to evaluate the dependence of priming upon microbial communities that either have or do not have a consistent source of "primable" C derived from rhizodeposition. Following injections of a total 100 ml of 290 μM sucrose solution into an unspecified collar-area in forest soils, Göttlicher et al. (2006) observed positive priming in areas which received regular inputs of labile C from rhizodeposits (i.e., ungirdled plots) and negative priming in soils without regular inputs of labile C (i.e., girdled plots). The authors interpreted these results to demonstrate the dependence of priming on the availability of "primable C," showing that in the absence of regular labile C inputs to soil, the priming effect following additional substrate amendments was negative, hinting at a switchover from the metabolism of SOM to sucrose, known as preferential substrate utilization (Göttlicher et al., 2006).

More explicit consideration of microbial communities using molecular methods has also shown that the diverse members of the soil community do not participate equally in the

uptake and mineralization of C additions. In a $^{13}CO_2$ pulse-chase labeling of annual ryegrass Butler et al. (2003) observed higher labeling of fungal phospholipid fatty acid (PLFA) biomarkers in soil than other PLFA biomarkers, suggesting that fungi may play a disproportionate role in the uptake of rhizodeposits. Similar results were also observed in a priming experiment utilizing multiple substrates in low doses performed on forest soils receiving long-term carbon input manipulations where fungi appeared to not only be relatively more abundant in soils with roots and extra wood additions compared to soils without any regular C inputs but that fungi PLFA biomarkers also appeared to be disproportionately labeled following ^{13}C-substrate additions (Brant et al., 2006b). Using soils from the same research plots as were used in our study, Brant et al. (2006b) suggested that the various priming responses observed following small (~50 μg substrate-C g^{-1} dry soil) substrate additions may be due to underlying differences in fungal to bacterial ratios found in soils with and without regular carbon inputs from roots and litter following selective experimental exclusion of these carbon inputs for seven years. In a companion study comparing microbial communities across the Detrital Input and Removal Treatment (DIRT) plots at three different sites, Brant et al. (2006a) found that the presence or absence of roots produced distinct microbial communities among DIRT treatments and that even with significant seasonal variations in the forest soils of the HJ Andrews research plots in central Oregon, significant differences persisted between soils with and without roots across time although the PLFA markers responsible for those differences also changed over time. A recent examination of the effects of roots on the turnover of SOM using Ponderosa pine and Fremont cottonwood trees during a 395-day greenhouse study also confirmed that the presence of tree roots created persistent priming effects in soils and that the inclusion of roots resulted in a net loss of C from soils compared to unplanted soils incubated under similar conditions (Dijkstra et al., 2007).

Examination of the rapid response of the microbial community to substrate additions using more discriminating molecular methods has revealed that the respiratory response in soils may be strongly linked to rapid shifts in the activity of specific organisms (Cleveland et al., 2007). By measuring small-subunit ribosomal RNA (SS-rRNA)genes in soils following

additions of dissolved organic matter (~225 μg DOM-C g^{-1} dry soil), Cleveland et al (2007) found that the increases in respiration following DOM additions corresponded to decreasing abundance of SS-rRNA genes of Acidobacteria and increasing abundance from Firmicutes and Gammaproteobacteria (which were not even detected in the unamended samples) within 12 hours of substrate additions. Based on the results summarized here, it would be expected not only that specific members of microbial communities drive glucose uptake and mineralization in soils, but also that the composition of those communities may determine the size and direction of priming responses.

Glucose Turnover and Modeling in Soils

The turnover and persistence of simple sugars in soil has been studied using a variety of methods and has produced varied estimates and turnover times. Though the turnover of sugars in soils has historically been considered to result in rapidly-diminished or transient soil C pools, recent studies examining the molecular characteristics and derivatives of SOM suggest that the average age of sugars found in soil using long-term incubations is comparable to the average age of bulk soil C (Gleixner et al., 1999; Gleixner et al., 2002; Derrien et al., 2006). Derrien et al. (2006) found average ages for glucose at 41 years in bulk soil compared to total SOC which had an average age of 58 years, and that with decreasing particle sizes, ages of both total SOC and glucose increased at approximately the same rate. Gleixner et al. (2002) found no relationship between supposed recalcitrance of pyrolysis-derived C compounds in soils and mean ages, suggesting that the unexpectedly high ages of N-containing compounds (49 years) and polysaccharides (54 years) reveal C recycling and physical protection from degradation may be more relevant factors governing the formation and turnover of SOM.

Targeting the microbial component to generate models of polysaccharide cycling in soils, Derrien et al. (2007) utilized one-time additions of ^{13}C-labeled glucose (400 μg C g^{-1} dry soil), glycine (200 μg C g^{-1} dry soil), cellulose (800 μg C g^{-1} dry soil), and wheat straw (800 μg C g^{-1} dry soil) in levels corresponding to expected natural addition rates over a series of incubations lasting three months or one year. Tracing the fate of glucose-derived C during

the one-year incubations of one soil revealed that the amount of C derived from labeled glucose in soils decreased by 50% over the first three days of incubation, to 27% of initially added substrate-C by six months, and that after one year of incubation, 18% of the C initially added as glucose remained in the SOM (Derrien et al., 2007). Modeling the dynamics of a variety of neutral sugars in the soils during these yearlong incubations, Derrien et al. (2007) isolated two distinct microbial metabolite pools, termed labile and preserved, which each followed first-order decay kinetics and determined that after six months of incubations following substrate additions, the profile of the various polysaccharides in soils did not change for the remainder of the incubation, suggesting preservation via physical protection against chemical degradation of these C compounds. The decay curve responses of glucose metabolism in both short and long-term studies is commonly fit using two pools, consistent with the interpretation that glucose respiration occurs through at least two separate metabolic pathways and that a non-trivial amount of glucose added to soils may cycle through a pool with relatively long turnover times (Brant et al., 2006b; Derrien et al., 2007; Hill et al., 2008).

Thesis Objective and Hypotheses:

Given the uncertainty in isolating microbial biomass from enzyme-mediated responses to C additions, I wanted to directly evaluate increasing enzyme activities as a potential mechanism for priming without producing biomass growth effects. By utilizing the DIRT plots at the HJ Andrews experimental forest, which are in an old-growth stand on the western side of the Cascade Range in central Oregon, I tested the responses of different microbial communities to glucose additions in relation to their long-term C availability. Because part of the major interest for this study was in the response of a natural, or relatively undisturbed, soil system to substrate additions, I conducted this experiment on site at the DIRT plots rather than through lab incubations of DIRT soils.

Through the addition of small (100 μg C g^{-1} dry soil) glucose additions to soils, I aimed to minimize any biomass growth responses to substrate additions and characterize the changes in enzyme activities as a function of DIRT manipulation and observed priming

effects. Consistent with the findings of Göttlicher et al. (2006), I expected to find positive priming in plots with roots and neutral or negative priming in plots where root inputs had been excluded for the past ten years. I hypothesized that the potential activities of phenol oxidase and peroxidase (lignolytic enzymes) would increase in relation to observed positive priming and that β-glucosidase activities would decrease following glucose additions due to the expectation that increasing the product of this enzymatic process would make production of this enzyme less beneficial. I expected that a modeling of the decay of glucose-derived C in the form of CO_2 would be best described by a two-pool model and that small but persistent amounts of glucose-derived C would remain in soils throughout the duration of a field experiment lasting approximately three weeks.

Table 1.1: Results of Studies Evaluating Enzyme Activities Relating to Priming

Substrates Added (μg substrate-C g^{-1} dry soil)	**Significant Enzyme Effects**	**Non-significant Enzyme Effects**	**Source**
glucose (300)	alkaline phosphatase (sandy and clayey soils), urease (clayey soil only)	protease, acid phosphatase, phosphodiesterase	Renella et al., 2007
glutamate (300)	alkaline phosphatase (sandy and clayey soils), acid phosphatase (sandy soil only), phosphodiesterase (clayey soil only)	urease, protease	
citrate (300)	acid phosphatase (sandy and clayey soils), alkaline phosphatase (sandy and clayey soils), urease (clayey soil only), phosphodiesterase (sandy and clayey soils)	protease	
oxalate (300)	acid phosphatase (sandy soil only)	protease, alkaline phosphatase, urease, phosphodiesterase	
acetate (8000)	--	β-glucosidase	Allison et al., 2005
acetate (8000)+ N*	β-glucosidase, acid phosphatase	--	
acetate (8000)+ celluloseP (?) + N	β-glucosidase	acid phosphatase	
acetate (8000) + P	--	glycine aminopeptidase	
acetate (8000) + collagen (?) + P	glycine aminopeptidase, acid phosphatase	--	
cellulose (8,000)	--	β-glucosidase	
cellulose (8000)+ N + P	β-glucosidase	--	
celluloseP (?)	--	β-glucosidase, acid phosphatase	
cellulose (8000) + P + N	β-glucosidase	--	
collagen (?)	acid phosphatase	glycine aminopeptidase	
natural tree exudates, girdling experiment (?)	β-xylosidase	β-glucosidase, α-glucosidase, β-D-cellobiosidase, N-acetyl-β-glucosaminidase, leucine aminopeptidase, acid phosphatase, phenol oxidase, peroxidase	Weintraub et al., 2007

Table 1.1: Results of Studies Evaluating Enzyme Activities Relating to Priming (continued)

Substrates Added (μg substrate-C g^{-1} dry soil)	**Significant Enzyme Effects**	**Non-significant Enzyme Effects**	**Source**
glucose (400) + KH_2PO_4	soluble and total protease	--	Asmar et al., 1994
glucose (800) + KH_2PO_4	soluble and total protease	--	
glucose (300)	phosphodiesterase, alkaline phosphomonoesterase	acid phosphomonoesterase	Renella et al., 2006a
glutamic acid (300)	phosphodiesterase, acid and alkaline phosphomonoesterase	--	
citric acid (300)	phosphodiesterase, alkaline phosphomonoesterase	acid phosphomonoesterase	
oxalic acid (300)	--	phosphodiesterase, acid and alkaline phosphomonoesterase	
mixture (300)	acid phosphomonoesterase	phosphodiesterase, alkaline phosphomonoesterase	
milled ryegrass (10)	acid and alkaline phosphomonoesterase	--	Renella et al., 2006b
glucose (3000)	phosphatase, urease, casein-hydrolyzing enzymes	--	Nannipieri et al., 1983
ryegrass (3770)	phosphatase, urease, casein-hydrolyzing enzymes	--	
King's agar* (1000)	soluble and total protease	--	Asmar et al., 1992
King's agar (1000) + glucose (600)	soluble and total protease	--	
King's agar (1000) + glucose (1200)	soluble and total protease	--	
glucose (3000)	proteases (casein, benzyloxycarbonylphenylalanyl leucine, benzoylarginine amide), phosphatase, dehydrogenase	--	Ladd et al., 1973

Table 1.1: Results of Studies Evaluating Enzyme Activities Relating to Priming (continued)

Substrates Added (μg substrate-C g^{-1} dry soil)	**Significant Enzyme Effects**	**Non-significant Enzyme Effects**	**Source**
starch (178)	xylosidase (oak and grassland soil), cellbiohydrolase and α-glucosidase (oak soil only)	α-glucosidase (grassland soil), β-glucosidase, cellbiohydrolase (grassland soil), galactase, n-acetylglucosaminidase, phenol oxidase, galactase	Waldrop et al., 2004
vanillin (250)	--	α-glucosidase, β-glucosidase, galactase, cellobiohydrolase, n-acetylglucosaminidase, , phenol oxidase	
pine litter (12000)	cellobiohydrolase and α-glucosidase (oak soil only)	β-glucosidase, cellobiohydrolase and α-glucosidase (grassland soil), galactase, n-acetylglucosaminidase, , phenol oxidase	
xylose (156)	--	α-glucosidase, β-glucosidase, galactase, cellobiohydrolase, n-acetylglucosaminidase, , phenol oxidase	

*Notes: In the Allison and Vitousek, 2005 study, N represents ammonium chloride, P is sodium phosphate, and celluloseP is cellulose phosphate. Values for the amount of C added in the forms of celluloseP and collagen were not given. For Asmar et al., 1992, values for King's agar represent total amount of substrate (rather than substrate C) added per gram of dry soil.

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Chapter 2:
Carbon Cycling and Priming of Soil Organic Matter Decomposition in a Forest Soil Following Glucose Additions

David D. Diaz

Introduction:

Globally, soils comprise the largest biologically active terrestrial carbon (C) pool, and changes in the turnover of C in the form of soil organic matter (SOM) may hold important ecological and environmental consequences (Janzen, 2004; Lal, 2004). Observations of increased soil CO_2 efflux under elevated atmospheric CO_2 and the potential for positive feedbacks of SOM turnover relating to changes in CO_2, soil moisture, or temperature regimes globally elevate the need for research on the mechanisms governing the formation and turnover of stable SOM (Cheng, 1999).

Though considerable effort has been focused on quantifying the fluxes and pool sizes of soil C in general, some of the fundamental mechanisms regarding the creation, persistence, and turnover of SOM are currently being reconsidered in light of recent studies utilizing new technologies (Marschner et al., 2008). Changes in the turnover of SOM following additions of organic substances through natural rhizodeposition, application of fertilizers and experimental substrates is known as priming, and has been defined as "strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil" (Kuzyakov et al., 2000). The most common mechanisms cited for SOM priming include increased degradation of SOM relating to enhanced SOM-degrading enzyme production and the turnover of microbial biomass carbon either in the form of intracellular C reserves or through the death and turnover of entire microbial cells (Kuzyakov et al., 2000).

Studies considering the sources of primed C have shown conflicting results. Though a growing number of studies support the hypothesis that MBC may be the sole source of primed C following labile C additions, there have also been studies showing more C arising from the priming of SOM than is contained in the MBC pool (Dalenberg et al., 1981; Dalenberg et al., 1989; Hamer et al., 2005). Though many studies have considered the relationship between exocellular enzyme activities and SOM priming, the results have been inconsistent and may be complicated by the fact that many of the experiments were using C additions that either are far outside of natural deposition rates or are multiple times the size of the MBC pool, making the isolation of enzyme responses from growth of microbial biomass impossible (Table 1.1)

Investigation of the response of diverse microbial communities to labile C additions has suggested that the presence or absence of root inputs to soil may contribute to significant differences in the community composition and yield significantly different priming effects (Brant et al., 2006a; Brant et al., 2006b; Göttlicher et al., 2006). The expectation that the availability of primable C relating to C input manipulations make long-term study sites such as the Detrital Input and Removal Treatments (DIRT) ideal for evaluating the contribution of various C inputs to the "primability" of different soils. Positive priming has been indirectly demonstrated in the field at the DIRT experimental plots in an old-growth coniferous forest in the Pacific Northwest following doubling of litter inputs, and considered directly through lab incubations of DIRT soils (Sulzman et al., 2005; Brant et al., 2006b).

Through field measurements of priming responses following small additions of ^{13}C-labeled glucose with the intent of minimizing biomass growth responses, I designed this study to document and evaluate the priming responses of diverse soil microbial communities and determine potential mechanisms governing observed priming effects. By measuring the activities of three SOM-degrading enzymes and changes in size and contribution of glucose-derived C to microbial biomass over the course of the experiment, I aimed to directly assess the relationship between labile C additions, enzyme degradation of SOM, and the potential sources of primed soil C. Turnover kinetics and microbial utilization of glucose were calculated by tracing and modeling the pools and fluxes of glucose-derived C through soil and gas pools.

Methods:

Study Site:

This study was conducted at the Detritus Input and Removal Treatments (DIRT) site at the H. J. Andrews Experimental Forest (HJA), Oregon (44°15′ N, 122°10′ W, 531 m elevation). Plant litter inputs have been chronically manipulated on these plots since 1997. The DIRT site was established in an undisturbed old-growth Douglas-fir (*Pseudotsuga menziesii*) – western hemlock (*Tsuga heterophylla* stand). Western red cedar (*Thuja plicata*) and vine maple (*Acer circinatum*) are present in lower abundances at the site. Four DIRT

manipulations were utilized for this experiment and are summarized in Table 2.1. There are three replicate DIRT plots per treatment type. Mean annual temperature at HJA headquarters is 8.7°C and mean annual precipitation is 2370 mm yr^{-1} (1973-2002). Over 70% of the precipitation typically occurs between November and March.

Priming Subplots:

Within each of the 12 DIRT plots used, one set of paired subplots were established. Each subplot was 30×30 cm square with a 10 cm diameter soil respiration collar installed at the center. All subplots received a one-time round of 16 evenly-spaced 30 ml injections to a depth of 10 cm. Multiple-port needles were inserted to a depth of 10 cm and slowly pulled upward during injection to evenly disperse the solution. One subplot per pair received injections of deionized water; the other subplot of the pair received injections of ^{13}C-labeled glucose solution. The glucose solution used had a concentration of 3.84g glucose/L and was enriched to 9.43 Atom% (7850‰). These injection amounts correspond to roughly 100µg glucose-C per g of soil (683 mmol C m^{-2}). All subplots received three injections within the soil respiration collar. All injections were performed on the morning of September 10, 2007 (day 0).

Soil Collection:

Soil Samples were collected prior to solution injections on September 10 (day 0), and again on September 11 and 20, 2007 (days 1 and 10). Soil samples were collected using 2 cm Oakfield corers to a depth of 10 cm. Between four and six cores (depending upon amount of soil removed) were pulled and composited per soil sampling. Soils were placed in a cooler and transported back to Oregon State University for processing each evening following collection. Day 0 samples were collected from immediately outside the 30×30 cm subplots. Day 1 soils were collected from the inside corners of each subplot, and day 10 soils were collected from the inside edges of each subplot. Soil samples were sieved to 2 mm.

Enzyme Assays:

Potential enzyme activities of β-glucosidase, phenol oxidase, and peroxidase were measured on soils from each collection (days 0, 1, and 10). Enzyme assays were performed the day after each field sampling. Soils for enzyme assays were refrigerated overnight at 4°C following collection. Enzyme assays were performed using the slurry method and followed modified protocols from Sinsabaugh et al. (1999). Briefly, 60 ml of DI-H_2O were added to 6 g of sieved soil and mixed vigorously on a stirrer plate. During mixing, 1-ml aliquots were withdrawn from the slurry and pipetted into separate vials for each assay. A 5-ml aliquot of slurry was also withdrawn and pipetted into a pre-weighed tin boat and dried in an oven at 45°C for 48 h to determine the mass of soil per volume of slurry. Three 1ml aliquots were used from each subplot per assay, one for a soil blank (soil + buffer) and two replicates which received enzyme substrate (soil + buffer + substrate). 1-ml of enzyme substrate was added to all vials except soil blanks. Samples were incubated in a 30°C water bath for one hour for all assays. See Table 2.2 for enzyme reagents used. Complete protocols can be found in Appendix A. Soil solutions were transferred to microcentrifuge tubes and centrifuged at 13,000 rpm (15,700 g) for 2 min. 200 µl aliquots of the supernatant were pipetted into 96-well plates and absorbance was read at 410 nm for β-glucosidase and 460 nm for phenol oxidase and peroxidase using a plate reader (SPECTRAmax 190, Molecular Devices Corporation, Sunnyvale, CA). A standard curve was created for *p*-nitrophenol concentrations for β-glucosidase activities. A micromolar extinction coefficient of 1.6 EC μmol^{-1} was used for calculation of the quantity of dihydroindole–quinone–carboxylate released in phenol oxidase and peroxidase assays (Sinsabaugh et al., 1999). Potential enzyme activities for each subplot are reported in this study using the means of the two replicate samples per assay.

Microbial Biomass:

Biomass fumigation was performed according to the standard chloroform fumigation method under vacuum (Vance et al., 1987) as modified by Bruulsema and Duxbury (1996) for ^{13}C analysis. Biomass determinations were made on all soils from days 0, 1, and 10. Following sieving, a 10 g (wet weight) sample of soil from each subplot was placed on a

shaker for 1 h with 20 ml of 0.05M K_2SO_4 and then filtered using Whatman #40 filter papers. Another sample was fumigated for 24 hours prior to extraction using ethanol-free chloroform. Four 0.5-ml aliquots of filtered extracts were pipetted onto acetone-rinsed tin squares (37 mm×37 mm) (Environmental Microanalysis, Manchester, MA) and dried at 45°C for 4 hours after addition of each aliquot. Tins were then balled up and analyzed for C content and $\delta^{13}C$ with using a PDZ Europa 20/20 IRMS. A K_C of 0.45 was used to convert chloroform flush C values into MBC (Wu et al., 1990). The following equation was used to determine the $\delta^{13}C$ value of MBC:

$$\delta^{13}C_{MBC} = \frac{\left(\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{unfum} \times C_{unfum}\right)}{C_{fum} - C_{unfum}}$$

Soil Respiration Rates:

Soil CO_2 efflux rates were measured from 12:00 pm to 3:30 pm on days 0 through 4, 7, 9, and 22. Contrary to the soil sampling regimen on day 0 which occurred before injections, gas sampling was performed 2-3 hours following injections. Soil CO_2 efflux rates were measured with a portable infrared gas analyzer (LI-6250, LI-COR Inc., Lincoln, NE) incorporated into a photosynthesis system (LI-6200), and attached to a closed, dynamic soil respiration chamber (LI-6200-09) designed for use with the LI-6200 (Norman et al., 1992). For each respiration measurement the soil respiration chamber was placed onto a 10-cm diameter polyvinyl chloride (PVC) respiration collar. The PVC collars were installed to a depth of 2 to 5 cm one day prior to injections (September 9, 2007). A foam gasket around the respiration chamber was used to form an airtight seal with the collar. Air in the chamber was partially scrubbed below ambient levels before beginning readings, and allowed to increase to just above ambient concentrations during measurements. CO_2 concentrations were measured for every 5 ppm increase for a total of three measurements. The data presented here are the output from the second of the three measurements.

$\delta^{13}C$-CO_2 of Soil Efflux:

Gas samples were collected for determination of $\delta^{13}C\text{-}CO_2$ of soil efflux on days 0 through 4, 7, 9, and 22. Gas samples were collected approximately 15 to 30 minutes after respiration measurements by placing a cap over the respiration collars and allowing for CO_2 concentrations to build up over time. Four samples of the headspace gas were collected using a 20-ml syringe through a septum in the cap. Samples were collected at roughly 0, 6, 12, and 18 min. Following collection using the syringe, the gas samples were then injected (overpressure) into 12 ml exetainers (738W, Labco. Limited, High Wycombe, UK), which were previously filled with N_2 gas and then evacuated using a vacuum pump. These gas samples were transported back to Oregon State University and analyzed for C content and $\delta^{13}C\text{-}CO_2$ within 48 hours of collection using a PDZ Europa 20/20 isotope ratio mass spectrometer (IRMS, Cheshire, UK). The $\delta^{13}C\text{-}CO_2$ of soil efflux was determined using the Keeling plot method (Keeling, 1958).

% Glucose and % Priming Calculations:

The percentage of glucose-derived CO_2 in soil efflux was calculated using the following equation:

$$\%C_{glucose} = \left[\frac{(\delta_C - \delta_T)}{(\delta_C - \delta_{glucose})} \right] \times 100,$$

where δ_C is the $\delta^{13}C$ value of the CO_2 respired from the water-treated (control) soils, δ_T is the $\delta^{13}C$ value of the CO_2 respired from the corresponding glucose-treated soils and $\delta_{glucose}$ is the $\delta^{13}C$ value of the glucose solution injected on Day 0.

Changes in the turnover of SOM following glucose additions was described as %Priming, which was calculated using the following equation:

$$\%Priming = \left[\frac{\left(1 - \frac{\%C_{glucose}}{100}\right) R_T - R_C}{R_C} \right] \times 100,$$

where R_T is the CO_2 efflux rate measured for glucose-treated soils and R_C is the CO_2 efflux rate for the corresponding water-treated soils (control). Thus, %Priming<0 indicates a

decrease in the efflux of SOM-derived CO_2 (negative priming) and %Priming>0 indicates an increase in the efflux of SOM-derived CO_2 (positive priming) due to glucose amendments.

Cumulative SOM-derived CO_2 flux and cumulative SOM-priming were determined by trapezoidal integration of lines connecting daily data points of flux rates. Changes in flux rates were assumed to change linearly between measurements and hourly flux rates were calculated based on linear changes between neighboring points. Missing data points were based on linear interpolation between neighboring data points. In cases where there were no neighboring points to interpolate between (i.e., in the case of missing data on day 0 or 22) the cumulative curves were either calculated beginning with first available data or truncated at the last measurement.

Glucose pools and fluxes

The efflux of glucose-derived CO_2 was modeled using two exponential decay models in SigmaPlot (Systat Software Inc., Point Richmond, CA). The first model used was a simple single exponential model:

$$F_{glucose\text{-}CO2} = G_0 \cdot e^{-kt},$$

where $F_{glucose\text{-}CO2}$ is the flux per area of glucose-derived CO_2 (mmol CO_2 m^{-2}) at time, t (d), G_0 is the initial efflux rate of glucose-derived CO_2 at t_0, and k is a decay rate constant (d^{-1}). The second model used was a compartmental model (Fig. 2.1). CO_2 derived directly from glucose was modeled as:

$$F_G = G \cdot k_1 = k_1 \cdot G_0 \cdot e^{-(k_1+k_2)t},$$

where F_G represents the flux of CO_2 coming directly from the glucose pool, G (mmol CO_2 m^{-2}), and k_1 and k_2 represent the decay rate constants for the fluxes shown in Figure 2.1. CO_2 derived directly from the microbial biomass pool (MBC) was modeled as:

$$F_{MBC} = MBC \cdot k_3 = \frac{k_3 \cdot k_2}{(k_3 - k_2 - k_1)} \cdot G_0 \cdot \left(e^{-(k_1+k_2)t} - e^{-k_3 t}\right),$$

where F_{MBC} represents the flux of CO_2 coming directly from the microbial biomass pool, MBC (mmol CO_2 m^{-2}), and k_3 represents the decay rate constant for the flux of C from MBC

to CO_2. Thus, the total equation used to model glucose-derived CO_2 efflux in the compartmental model was:

$$F_{glucose\text{-}CO2} = F_G + F_{MBC} = k_1 \cdot G_0 \cdot e^{-(k_1+k_2)t} + \frac{k_3 \cdot k_2}{(k_3 - k_2 - k_1)} \cdot G_0 \cdot \left(e^{-(k_1+k_2)t} - e^{-k_3 t}\right).$$

$$\text{Glucose pool, } G = G_0 \cdot e^{-(k_1+k_2)t}$$

$$\text{Microbial biomass pool, MBC} = \frac{k_2}{(k_3 - k_2 - k_1)} \cdot G_0 \cdot \left(e^{-(k_1+k_2)t} - e^{-k_3 t}\right)$$

$$\text{Yield coefficient} = \frac{k_2}{k_1 + k_2}$$

Statistical Analysis:

Determination of intercept values for Keeling plots was performed using ordinary least squares regression in S-Plus 6.1(Insightful Corp., Seattle, WA). Regressions with an r^2 of < 0.7 were inspected and if possible, had single outlier points removed. Those data from plots which had r^2 values < 0.7 following single outlier removal were excluded from further analysis. The r^2 values for regressions were typically high (0.95 ± 0.06 *SD*, *n*=185) and the $\delta^{13}C$-CO_2 values from Keeling plots with $0.7 < r^2 < 0.9$ (*n*=31) are consistent with Keeling plots from other collars with $r^2 > 0.9$ from the other replicate collars on the same sampling day. Treatment effects were evaluated using repeated measures analysis of variance (RM-ANOVA) using PROC MIXED in SAS 9.1 (SAS Institute Inc., Cary, NC). The RM-ANOVA was conducted using a first order autoregressive variance-covariance structure, degrees of freedom calculated using the Kenward-Rogers approximation. *A priori* planned multiple comparisons were made comparing glucose and water treatments within each DIRT plot for each sampling day and are presented with unadjusted *p*-values. Comparisons considering multiple different DIRT treatments were conducted using the Bonferroni correction to achieve a family-wise error rate of $\alpha = 0.05$. Curve fitting and determination of model parameters using respiration data was performed on log-transformed respiration rates using SigmaPlot 9.0/SigmaStat3.1 (Systat Software Inc., San Jose, CA). Data from one Control water-treated collar was excluded from all analysis due to outlier biomass and

respiration measurements based on installation of the collar onto a buried rotting log. The outputs from all RM-ANOVA models are presented in Appendix B.

Results:

Respiration and Glucose-Derived CO_2:

Injections of water and glucose produced a disturbance effect in efflux rates, with CO_2 efflux highest immediately following injections and decreasing over time (Fig. 2.2). Glucose additions produced a higher CO_2 efflux on the day of injection compared to water injections, but this difference disappeared by the following day, except in the case of No Input plots where glucose appeared to have a negative effect on CO_2 efflux beginning one day after injection. The differences between glucose-treated and water-treated respiration rates within each DIRT treatment were statistically significant only for the Control DIRT treatment on day 1 (p=0.021). The Control plots responded strongly to the glucose injection while the No Litter, No Roots and No Inputs plots produced a much smaller response to glucose injections.

The $\delta^{13}C$-CO_2 of soil efflux was highly labeled following glucose injections and decreased exponentially over time, leveling out at approximately 100‰ enrichment over natural abundance 22 days after injection (Fig. 2.3). The efflux of glucose-derived CO_2 followed an exponential decay that was more effectively modeled using a two-compartment model than with the single-exponential model (Fig. 2.4). The single-exponential model was biased to the turnover of the fast pool and failed to effectively model the tail end of measurements when glucose-derived CO_2 flux was low. The two-compartment model followed the tail end of the data more closely and only slightly under-predicted the early data points.

SOM-derived CO_2 and Priming:

Glucose additions produced positive average %Priming in Control and No Litter plots and negative average %Priming in No Roots and No Inputs plots which appeared to peak 2-3 days following injection and disappeared by day 4 (Fig. 2.5). The Control plots had a much

higher variability and magnitude of priming responses (both in terms of %Priming and efflux rate of primed CO_2) over time than did any of the other DIRT treatments. The magnitude of the %Priming response for all DIRT removal treatments (i.e., No Litter, No Roots, and No Inputs) were approximately equal, with positive and negative %Priming effects producing approximately 25% relative changes in the turnover of SOM-derived CO_2. The %Priming responses were typically not significantly different from zero except in the case of the Control plots for days 0, 2, and 22.

Comparison of SOM-derived respiration in glucose-treated collars to water-treated collars revealed that based on averages per DIRT treatment, only the Control plots had consistently higher SOM-derived respiration rates (i.e., positive priming) throughout the course of the experiment (Fig. 2.6). SOM-derived respiration rates are not normalized by the basal respiration of the water-treated collar as is the case in %Priming. This may help explain why on average, the No Litter DIRT treatment had positive %Priming for several days while still having a net deficit of SOM-derived CO_2 flux. More specifically, %Priming as an average of several collars can be swayed by comparatively small shifts in the proportional change of SOM-turnover for each pair of collars whereas SOM-derived CO_2 flux rates are not as susceptible because they are unadjusted flux rates. A negative relationship was found between the scale of the priming effect and the background respiration rates measured from water-treated collars for both %Priming and efflux rate of primed CO_2 (Fig. 2.7). Collar pairs with higher background respiration rates generally showed negative priming. This trend appeared to correspond only to the DIRT removal treatments (i.e., No Litter, No Roots, and No Inputs) and not to the Control DIRT plots.

Cumulative priming trends were highly variable from collar to collar within each treatment (Fig. 2.8). Cumulative primed CO_2 ranged widely across the collars (Table 2.3). Of the collars for which cumulative priming was calculated, one Control and one No Inputs collar primed enough CO_2 to develop a net loss of C from the soil. The observed cumulative priming of C was never calculated to be larger than the C contained in the microbial biomass at the beginning of the experiment. On average, priming in the Control plots was continuous and led to the release of more SOM-derived CO_2 in glucose-treated plots than from water-

treated plots (Fig. 2.9). On average, all other DIRT treatments produced less SOM-derived CO_2 over the course of the experiment from glucose-treated collars than from water-treated ones.

Microbial Biomass, DOC, and Bulk Soil:

There were no significant changes in biomass corresponding to glucose additions on any of the sampled days. Biomass decreased significantly during the experiment. Days 0 and 1 had significantly higher biomass than day 10 (p=0.013 and 0.040, respectively), but this trend was unrelated to glucose versus water additions and occurred in both treatment types (Table 2.4). The microbial biomass became increasingly enriched over the course of the experiment and corresponded to the uptake of 2 to 42% of added glucose by day 1 and 5 to 31% by day 10 (Table 2.5)

By day 1, the injected glucose had predominantly left the DOC pool in soil. The DOC pool was typically the smallest soil reservoir of glucose-C when measured directly and usually contained less than 10% of the added glucose-C one day following injections and less than 5% ten days after injections. The microbial biomass pool was comparable in size to a non-extractable (after 1 hr with 0.05M K_2SO_4) and persistent pool of glucose-C in the soil. The amount of glucose-C in microbial biomass tended to increase from day 1 to day 10, but did not appear to be related to DIRT manipulation type.

The injections of glucose and water produced similar increases in the soil moisture content of the soils which dissipated over the course of the experiment. Gravimetric water content for day 1 (25.0% ± 0.67% SE) was slightly elevated over day 0 (23.0% ± 0.46% SE) and day 10 (22.9% ± 0.91% SE).

Enzyme Activities

None of the enzymes measured showed significant or consistent changes in potential enzyme activities in relation to glucose amendment (Figs. 2.10 and 2.11). RM-ANOVA for the potential activities for β-glucosidase revealed that Day of collection was the only

statistically significant factor ($p<0.001$). β-glucosidase activities were lower on day 10 than on days 0 and 1. This effect showed up in both glucose-treated and water-treated soils.

RM-ANOVA for phenol oxidase showed significant effects for DIRT treatment ($p<0.001$), Day ($p<0.001$), a significant interaction between Glucose/Water*Day ($p=0.026$), and a marginally significant interaction between DIRT Treatment and Glucose/Water addition ($p=0.137$). Multiple comparisons of DIRT treatments reveal that phenol oxidase activities were typically higher in Control and No Inputs plots than in No Litter plots (adjusted $p= 0.011$ and 0.002, respectively). No Inputs plots were also found to have higher phenol oxidase activities than No Roots (adjusted $p= 0.011$). These multiple comparisons were for DIRT treatments in general, and no significant effects were found relating to the presence or absence of glucose additions. Similar to the pattern with β-glucosidase, phenol oxidase activities were also lower on day 10 than on days 0 and 1. This effect shows up in significant comparisons across days for the water-treated soils but not for glucose-treated soils, which likely drove the significant Glucose/Water*Day interaction. There was only one point through the course of the experiment where glucose-treated collars were statistically distinct ($p=0.003$) from water-treated collars. On day 1 for the Control DIRT treatment, phenol oxidase activities were lower in the glucose-treated collars than in the water-treated ones.

Day of collection was also a significant effect for peroxidase with activities on day 10 again being lower than days 0 or 1. DIRT Treatment ($p=0.090$) and Glucose/Water ($p=0.107$) were marginally significant effects in phenol oxidase RM-ANOVA. None of the multiple comparisons between DIRT treatments were statistically significant following Bonferroni adjustments, and the glucose-treated versus water-treated contrast which was marginally significant ($p=0.107$) showed lower peroxidase activities for glucose-treated soils than for water-treated soils. The ANOVA tables for all enzyme activities can be found in Appendix B.

Glucose Mass Balance and Decay Kinetics

Approximately 70% of added glucose-C was accounted for during measurement of soil and gas pools (Table 2.6). Partitioning glucose into different pools for mass balance revealed a significant amount of "missing" carbon (30-40%). The majority of this carbon is expected to correspond to respiratory flux after injections of glucose and water but prior to the commencement of gas measurements (2-3 hours). On average, total recovery of glucose-C was higher on day 10 than on day 1. Model-fitting performed on glucose-derived CO_2 flux rates yielded similar parameters for each of the DIRT treatments (Table 2.7). The behavior of the model pools following input of parameters derived from glucose-derived efflux showed the DOC or fast pool being completely depleted by approximately day 5 (Fig. 2.12). The contribution of the MBC or slow pool to glucose-derived CO_2 flux began quickly and started to plateau within two days following injections. After about 4 days, the majority of glucose-derived CO_2 in the model was derived from the turnover of the slow pool.

Discussion:

Respiratory Efflux Rates

The additions of glucose produced a short-lived increase in soil respiratory efflux for all DIRT treatments above any disturbance effects due to injection (Fig. 2.2). I suspect that there may have been a larger increase in soil respiratory activity which was missed between the injection of glucose and first respiratory measurements. Hill et al. (2008) found that 2 to 7% of added glucose was mineralized within a minute of addition, and that 10 to 15% was mineralized within an hour.

The decrease in total CO_2 efflux rates following glucose additions found in No Litter and No Inputs DIRT plots was unexpected and atypical. In a lab incubation using glucose additions at a level of 50 μg C g^{-1} dry soil on soil from the same DIRT plots, the difference in respiratory efflux rates between glucose-treated and control soils returned to zero within 3 to 4 days and stayed near zero for the remainder of the 14-day incubation (Justin Brant, unpublished data). Using glucose additions at 11.3 μg glucose-C g-1 dry soil, De Nobili et al. (2001) observed a 4-day lag prior to respiratory increase and then between days 9 to 14 appear to have documented reduced respiratory efflux from glucose-treated soils compared to

non-amended soils, while there did not appear to be a consistent decrease in total flux at additions of 34 μg glucose-C g^{-1} dry soil. Reports of negative priming effects are less common in the literature than are positive priming effects and typically invoke "preferential substrate utilization" whereby soil microorganisms reduce their consumption of comparatively recalcitrant SOM in favor of labile C added in substrate amendments (Cheng, 1996; Kuzyakov et al., 2000). Preferential substrate utilization does not imply that total respiratory efflux is reduced, but rather that the amount of SOM-derived CO_2 is reduced even though total CO_2 flux may be higher. Thus, the total reduction of respiratory activity would correspond to decreased microbial activity in general rather than preferential substrate utilization alone. In my experiment, the negative respiratory effects observed in No Inputs and No Litter plots persisted from 2 to 22 days following glucose injections, long after priming-related effects from such small additions would be expected to contribute to soil microbial activity.

Quantification of the Priming Effect

There is not a consistent method used for quantifying priming, and priming data are commonly reported in absolute and relative terms. Priming may be measured as proportional changes in the amount of SOM-derived CO_2 compared to soils without C additions (e.g., %Priming in this study), or in terms of absolute values of amount of Primed CO_2, cumulative primed CO_2 flux, or extra C mineralized (i.e., substrate-induced respiration). Though Kuzyakov et al. (2000) discuss potential advantages and disadvantages of the various priming quantification techniques, both absolute and relative methods may offer different and insightful views into the priming effect. %Priming values may be more sensitive to small, but potentially physiologically important changes in the metabolism of SOM. Thus, even small changes in SOM-derived CO_2 efflux may produce large %Priming values if the change in flux rates is large compared to the flux rate of the control or non-amended soils. Absolute measures of priming may provide more informative estimates for evaluation of net C balance following substrate additions, but are less sensitive to small changes in flux rates for soils that have lower efflux rates in general. The relationship between background respiration rates and priming responses demonstrated in this study (Fig. 2.7) suggests that soil of

different background activities do not respond the same way to substrate additions. Considering the potential biases of these quantification methods and the apparent relationship between larger background flux rates and increasingly negative priming rates, it would seem that small but potentially physiologically important positive priming effects could be missed if only absolute measures of priming are used or that ecologically significant fluxes of C could be missed if only relative measures of priming are used.

Observed Priming Responses

The priming responses observed in this study are comparable to those observed in a lab study performed using soils from the same DIRT plots. Twelve hours after glucose additions, Brant (unpublished data) observed priming rates of 70 to 165% in DIRT treatment soils. Several points of negative priming were observed in their experiment between days 2 to 4, but were always less that <20% of respiration rates of non-amended soils.

Relative priming values were positive in Control and No Litter DIRT plots and negative in No Roots and No Inputs plots (Fig. 2.5). One major difference between these two groups of treatments is the presence/absence of root inputs to soil. The %Priming data reported in relation to root inputs are consistent with those from a study following the addition of sucrose to soils after tree girdling. Göttlicher et al. (2006) added sucrose (100 g L^{-1}, as compared to 3.8 g glucose L^{-1} in this study) and found up to 60% increases in the turnover of SOM (positive %Priming) in non-girdled plots (i.e., plots with undisturbed rhizosphere systems) and 40 to 50% decreases in SOM-turnover (negative %Priming) in girdled plots, suggesting that that intact rhizomicrobial communities were able to produce positive priming effects whereas those without regular inputs of root exudates switched over to preferential substrate utilization rather than continuing to metabolize SOM. The presence of positive priming with intact root systems and negative priming where their exudation activity has been stopped suggests that reserves of "primable" C are quickly exhausted in soils after the cessation of root exudation caused by tree girdling. Microorganisms that regularly experience root exudation are therefore expected to develop a reserve of C which

can quickly be turned over following additional availability of labile C, consistent with the hypothesis of intracellular MBC as the source of primed C (Göttlicher et al., 2006).

The removal of C inputs rather than presence/absence of roots appeared to characterize the split between positive and negative priming responses when using absolute priming values. The Control DIRT treatment was the only one to consistently produce positive priming results for SOM-derived CO_2 efflux rates and for cumulative primed C whereas the No Litter, No Roots, and No Inputs treatments on average consistently produced less SOM-derived CO_2 from glucose-amended plots than from water-treated ones (Figs. 2.6 and 2.9). The removal of both litter and root C inputs to the soil each appear to induce negative priming responses compared to Control soils. These results conflict with those found by Brant et al. (2006b) who showed consistent positive priming in Control and No Inputs soils using lab incubations with glucose.

The direction and magnitude of the response to glucose additions was highly variable for individual pairs of collars. Different collars from all DIRT treatments were observed to produce positive and negative cumulative priming, except for Control plots, which only showed positive priming (Fig. 2.8 and Table 2.3). The amount of primed CO_2 produced over the course of the 22 days of gas sampling was always less than the starting pool of MBC at the beginning of the experiment. For most of the collars, there were also measured decreases in MBC over the course of the experiment that may also contribute to the observed priming effects. Thus, we are unable to reject the hypothesis that MBC is the source of primed C based on volume of C fluxes alone as others have (Hamer et al., 2005). It is not clear what led to the decrease of MBC in these soils, particularly given the fact that this decrease appears to occur in both glucose- and water-treated collars. This lack of a glucose-driven destruction of microbial biomass is consistent with Wu et al. (1993) who found a destructive effect of glucose at concentrations of 5000 μg glucose-C g^{-1} dry soil but not for a lower addition of 500 μg glucose-C g^{-1} dry soil.

Negative priming appears to be a characteristic response for soils without roots. The various priming responses of No Litter plots depending on quantification method make generalization of litter effects unclear. Plots which receive unaltered C inputs (i.e., Control

DIRT treatment) consistently produced positive priming effects. The high variability of each DIRT manipulation with only three replicates makes statistical inference considerably difficult, but general trends of priming support the interpretation of Göttlicher et al. (2006) that selective removal of C inputs of roots (and perhaps from litter as well) leave the extant microbial communities comparatively impoverished and less equipped to respond positively to labile C additions.

Glucose Amendments as a Disruption of the Status Quo

The relationship between background respiration rates and priming responses using both relative and absolute measures is a novel relationship in priming research. The negative correlation between background respiration rate and priming response held only for DIRT removal treatments (i.e., No Litter, No Roots, and No Inputs) and not for the Control DIRT treatment. The presence of this relationship only in removal treatments suggests that highly active soils with restricted C inputs may be disrupted by the addition of glucose such that microbial decomposition of SOM decreases. The fact that this relationship is strongest for No Inputs plots further supports that hypothesis that the extant microbial community following 10 years of DIRT manipulations has become specialized in the turnover of particular C pools in soil and that the addition of labile C may not induce increased respiratory activity, but may rather produce a significant disruption of the *status quo* resulting in an overall decrease of microbial activities.

The Role of Enzymes in Priming

There were no consistent enzyme changes supporting the hypothesis that SOM-degrading enzymes are responsible for the priming effect. In fact, on a few occasions, potential enzyme activities were significantly lower in glucose-treated soils than in water-treated ones. There were also no significant enzyme effects in relation to DIRT manipulations. The lack of consistent enzyme responses support the working hypothesis of Fontaine et al. (2003) that soil microorganisms do no adjust enzyme production in response to substrate availability. Results from this study suggest that soil microorganisms in this

system do not respond to differential availability of C related to DIRT manipulations and glucose additions by producing new enzymes. I suspect that it may be difficult to demonstrate the role of enzymes in priming because of the high levels of variability of potential enzyme activities in soil. Given high background variability, detection of changes may be possible only through larger sampling regimes with considerable effort aimed at determining satisfactory average activities for each replicate of a treatment. The high degree of replication necessary to achieve significance utilizing enzyme assays makes this potential valuable source of ecosystem information difficult to assess, particularly when considering short-lived effects such as priming of SOM.

Glucose Cycling, Mass Balance, and Kinetics

The amount of glucose-C found in MBC (Table 2.6) is at the lower end of a wide range of values found in other studies using similar glucose additions where anywhere from 20 to 80% of added glucose was found in MBC within 2 to 3 days after additions (van Veen et al., 1985; Bremer et al., 1994; Lundberg et al., 2001; Brant et al., 2006b). The amount of glucose-C recovered as CO_2 is slightly lower than that found in a lab incubation study using soil from the same DIRT plots but should reasonably be expected to be lower than from lab incubations in general due to the expected mineralization of glucose-C between glucose injections and commencement of gas measurements in this study (Brant et al., 2006b; Hill et al., 2008). The amount of glucose-C contained in both DOC and MBC is relatively small compared to the size of each pool, contributing only 5 to 10% of the C found in both DOC and MBC.

The yield efficiency, that is the amount of glucose converted into biomass related to amount mineralized to CO_2, for glucose metabolism has been quantified in a variety of ways across studies. Our yield coefficients calculated through modeling of glucose-derived CO_2 flux lie at the low end of reported ranges from 26 to 82% (Frey et al., 2001; Brant et al., 2006b). Based on these values, the microbes metabolizing glucose in all of the DIRT treatments measured in this experiment appear to be less efficient at converting glucose-C into MBC than expected from other studies and that more of the glucose-C is mineralized to CO_2 rather than incorporated into microbial cells. Lower yield efficiencies observed in this

field study compared to lab incubations of the same soils may derive from stricter nutrient limitations in the field as compared to sieved and homogenized soils used in lab incubation studies.

The mean residence times for glucose in the forms of DOC and MBC correspond to $(k_1+k_2)^{-1}$ and $(k_3)^{-1}$, respectively, yielding average turnover times of 1.1 d for DOC and 32 d for MBC. These values lie in between estimates of glucose turnover ranging from as fast as 30 s up to 41 yr in bulk soil (Derrien et al., 2006; Hill et al., 2008). These vast differences observed in these estimates are likely related to the timescale of incubations and methodologies for calculating turnover times. Kinetics of glucose turnover in short-term studies may be largely influenced by the rapid mineralization of most of the glucose-C whereas longer-term studies can be more influenced by the persistence of glucose-C prolonged via physical and chemical association with soil particles or through the recycling of intact glucose molecules (Derrien et al., 2006; Derrien et al., 2007).

Conclusions:

The priming responses of soils to glucose additions appear to be regulated by the availability of primable C. In soils that have received reduced C inputs from roots, consistent negative priming effects were found considering both relative and absolute measures. Soils receiving litter exclusions showed an inconsistent response to glucose-additions with positive %Priming responses suggesting that some of the soils may be physiologically triggered by glucose additions, but that these small changes in the turnover of SOM from already slowly respiring soils may be overwhelmed by the negative priming responses of more metabolically active soils. The negative relationship between background respiration rates and priming response for both relative and absolute measures is a new finding in this field of research and suggests that the activities of microorganisms that have adapted to environments with restricted C inputs may be significantly disrupted following labile C additions, leading to net reductions in the turnover of SOM.

The amount of C produced via positive priming effects was too small to rule out MBC as a sole source of primed C. This interpretation is further supported by the lack of

consistent changes of potential enzyme activities of SOM-degrading enzymes in relation to labile C inputs. The lack of DIRT treatment effects on enzyme activities also furthers the working hypothesis put forward by Fontaine et al. (2003) that soil microorganisms do not produce enzymes in response to substrate availability, but rather that these production events may correspond to phases of biomass growth. Despite the fact that priming cannot be directly related to the increased metabolism of NM-SOM, it seems unlikely that C-limited microorganisms in the soil considered in this study would maintain intracellular C reserves amounting to as much as 50% of MBC. The heterogeneity and variability of enzyme activities in soils makes demonstration of enzyme effects considerably more difficult than showing no significant effects. To further evaluate the role of exocellular enzymes in the priming of SOM, significant emphasis should be placed on achieving satisfactory sample sizes and reproducible assays for large numbers of samples. Though enzyme activities are often considered putative sources for priming of SOM, very few studies have directly measured their responses to C additions. In further research conducted along this line of study, it is important to try and isolate biomass growth effects from enzyme production if we are to make meaningful interpretation of priming effects observed following natural changes in C inputs to soils related to rising CO_2 and environmental change.

Figure 2.1: Glucose Pools and Fluxes

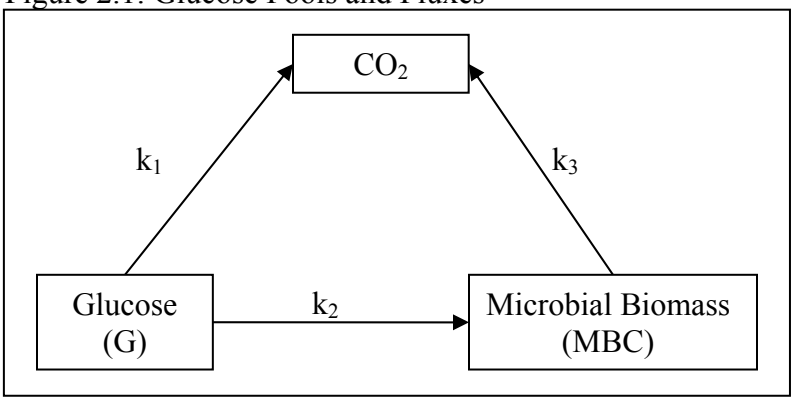
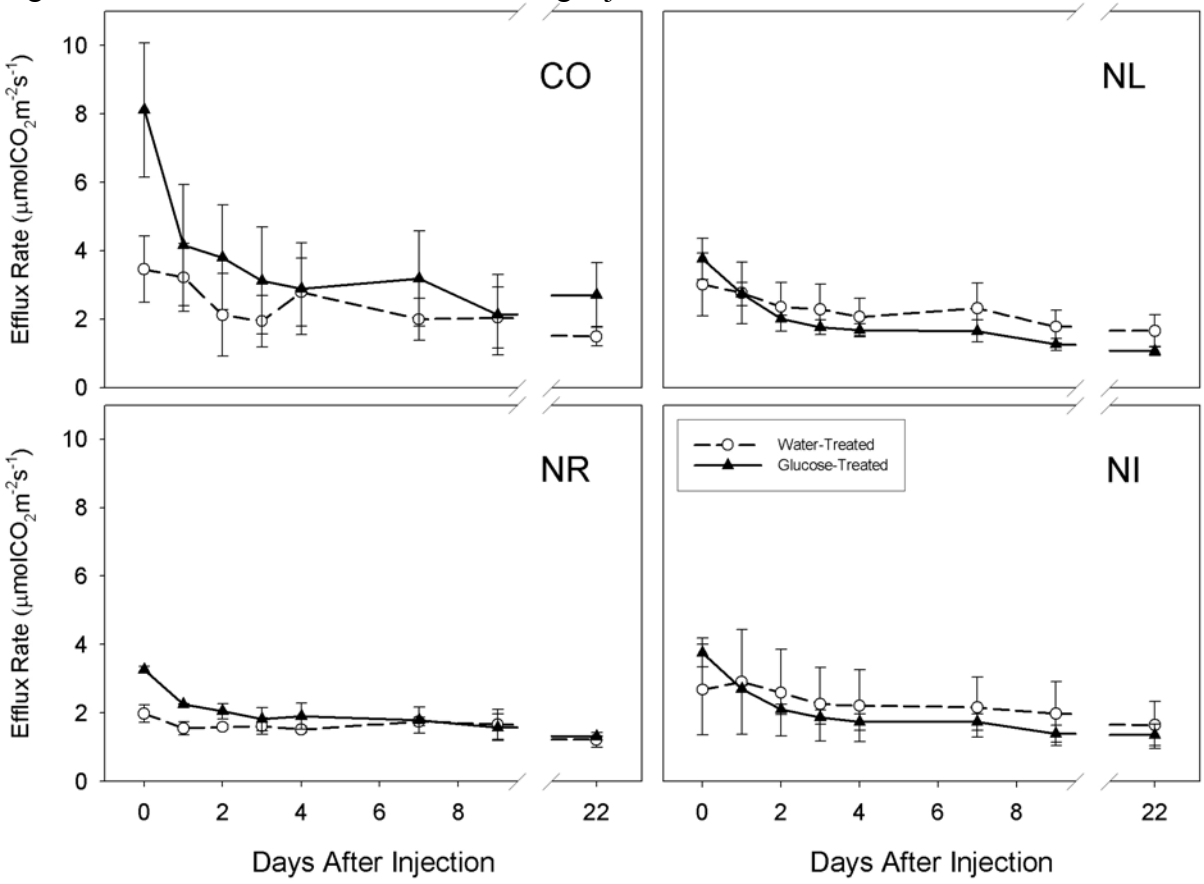
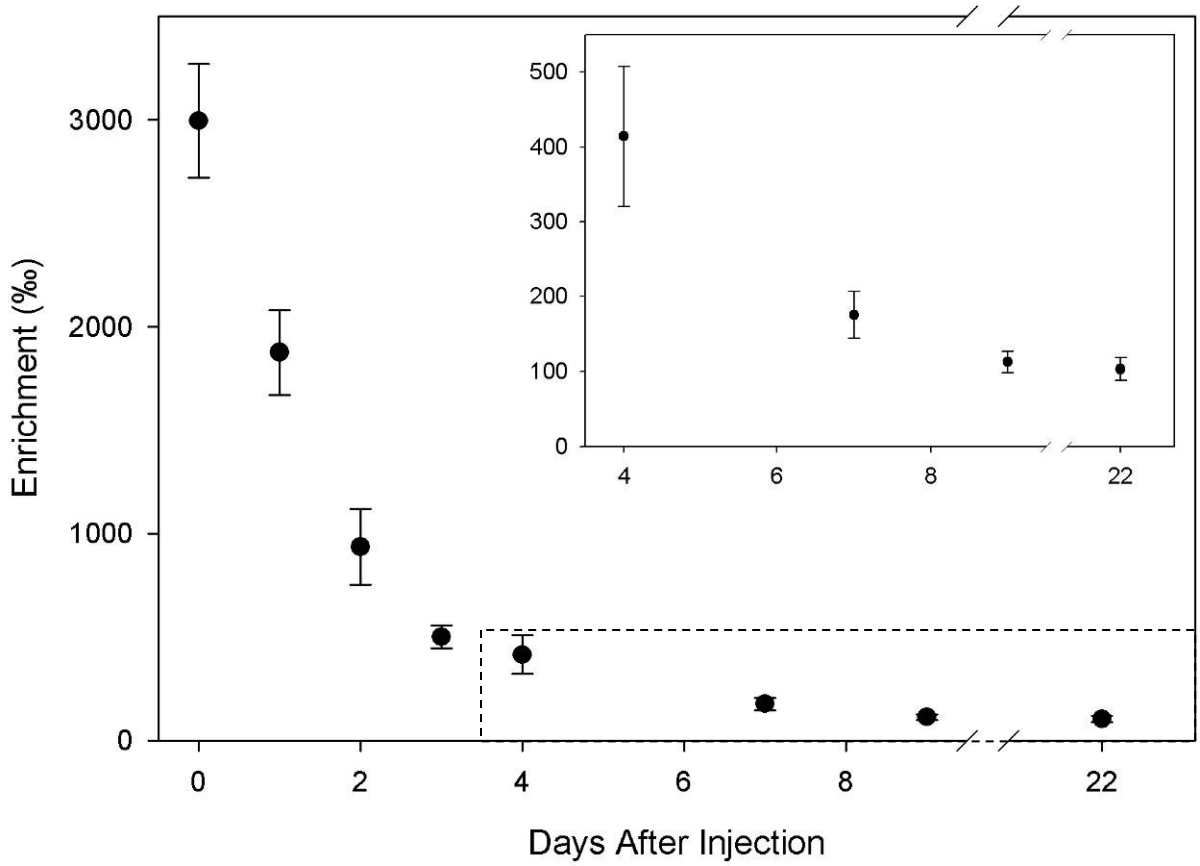


Figure 2.2: Soil CO_2 Efflux Rates Following Injections



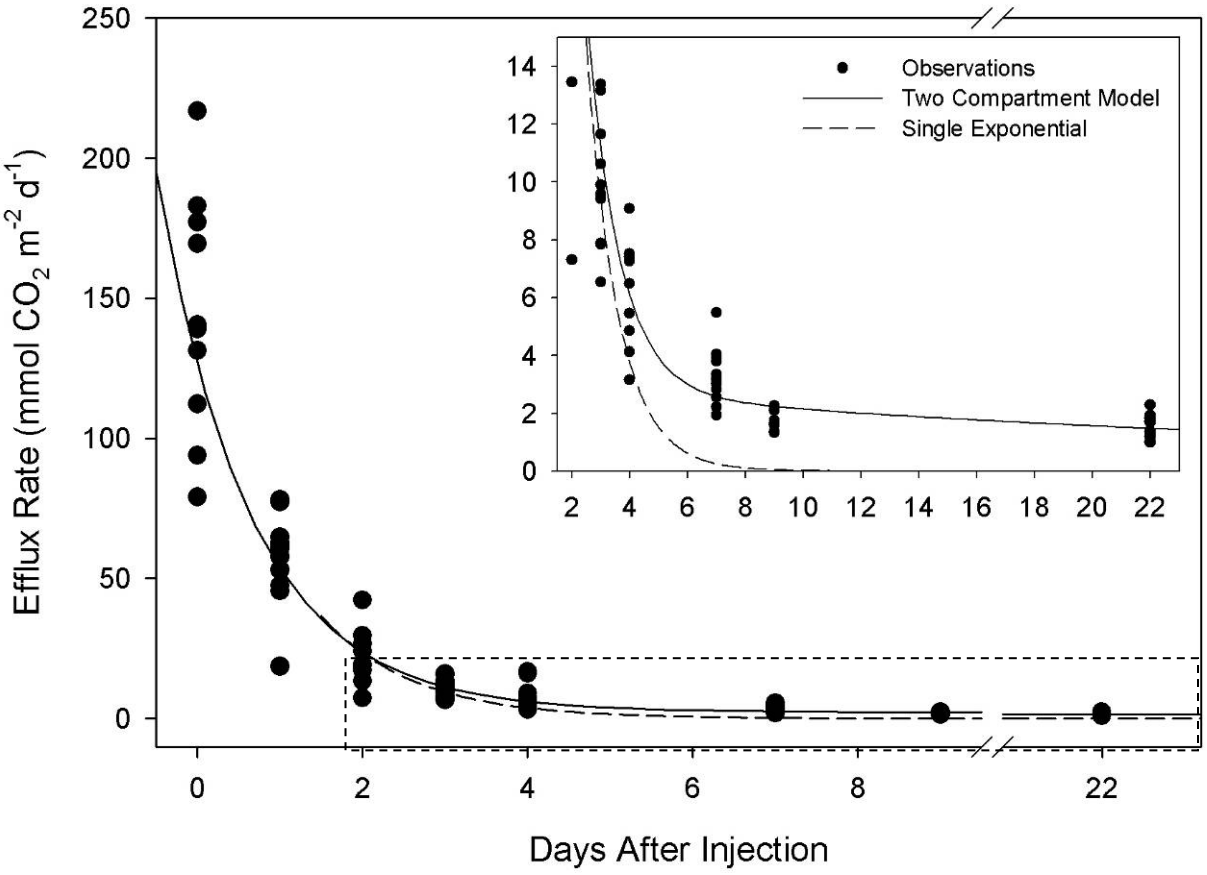
Notes: All graphs have the same x and y-axis scales. Error bars represent one standard error for collars from all three DIRT replicate plots (n=3), except for CO treatment where n=2. Black triangles and lines are for glucose-labeled collars, and white circles with gray lines are for water-treated collars. The two letter code in the top right corner is the DIRT manipulation type -- Control (CO); No Inputs (NI); No Litter (NL); No Roots (NR). There is a break in the x-axis between days 9 and 22. An asterisk (*) indicates a significant difference between water and glucose-treated rates ($p \leq 0.05$).

Figure 2.3: $\delta^{13}C\text{-}CO_2$ Enrichment



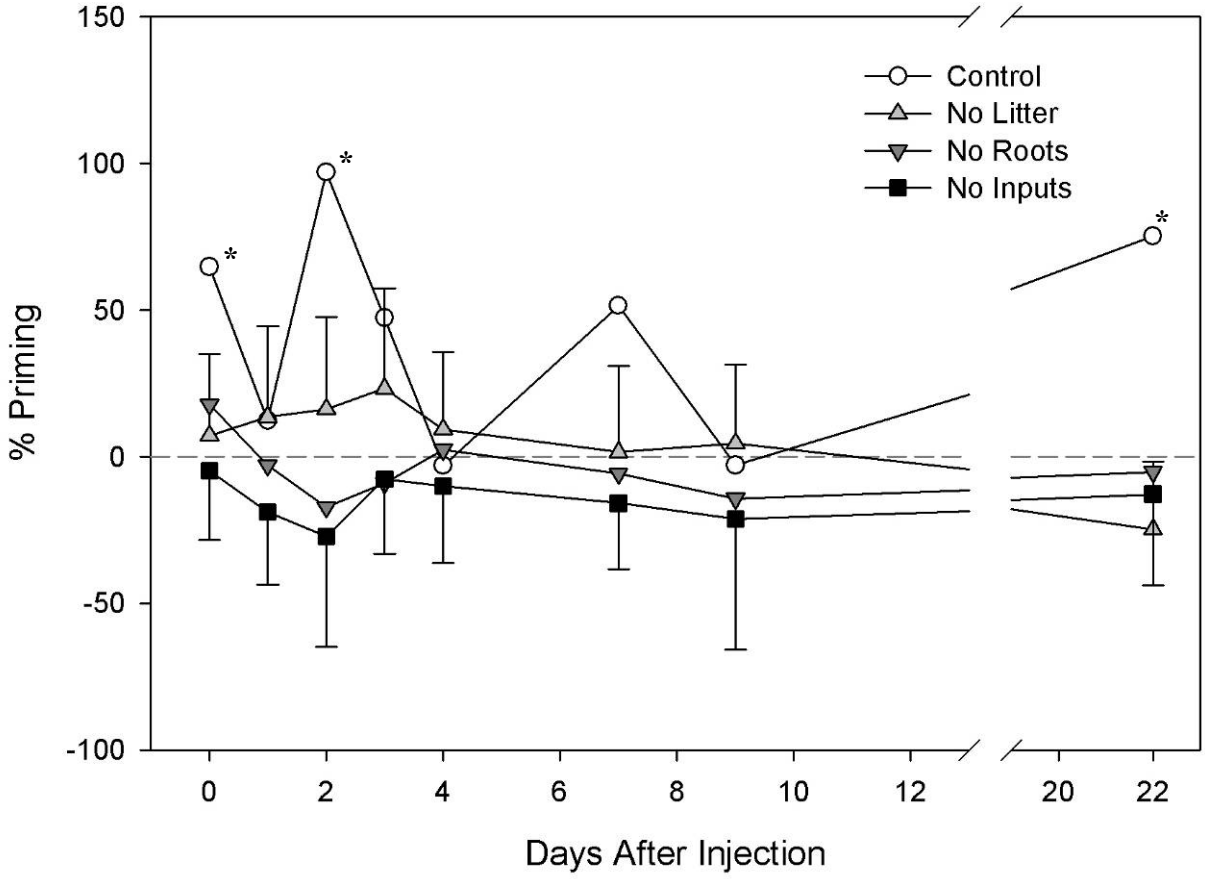
Notes: This graph displays the difference between the $\delta^{13}C\text{-}CO_2$ of glucose-treated collars compared to their paired water-treated collars for all collars from all DIRT manipulation types. The area inside the dashed line is magnified in the inset graph. Error bars represent one standard error for the isotopic enrichment for all of the paired collars (n=11). There is a break in the x-axis for both the large and inset graphs between days 9 and 22.

Figure 2.4: Glucose-Derived CO_2 Efflux and Modeling



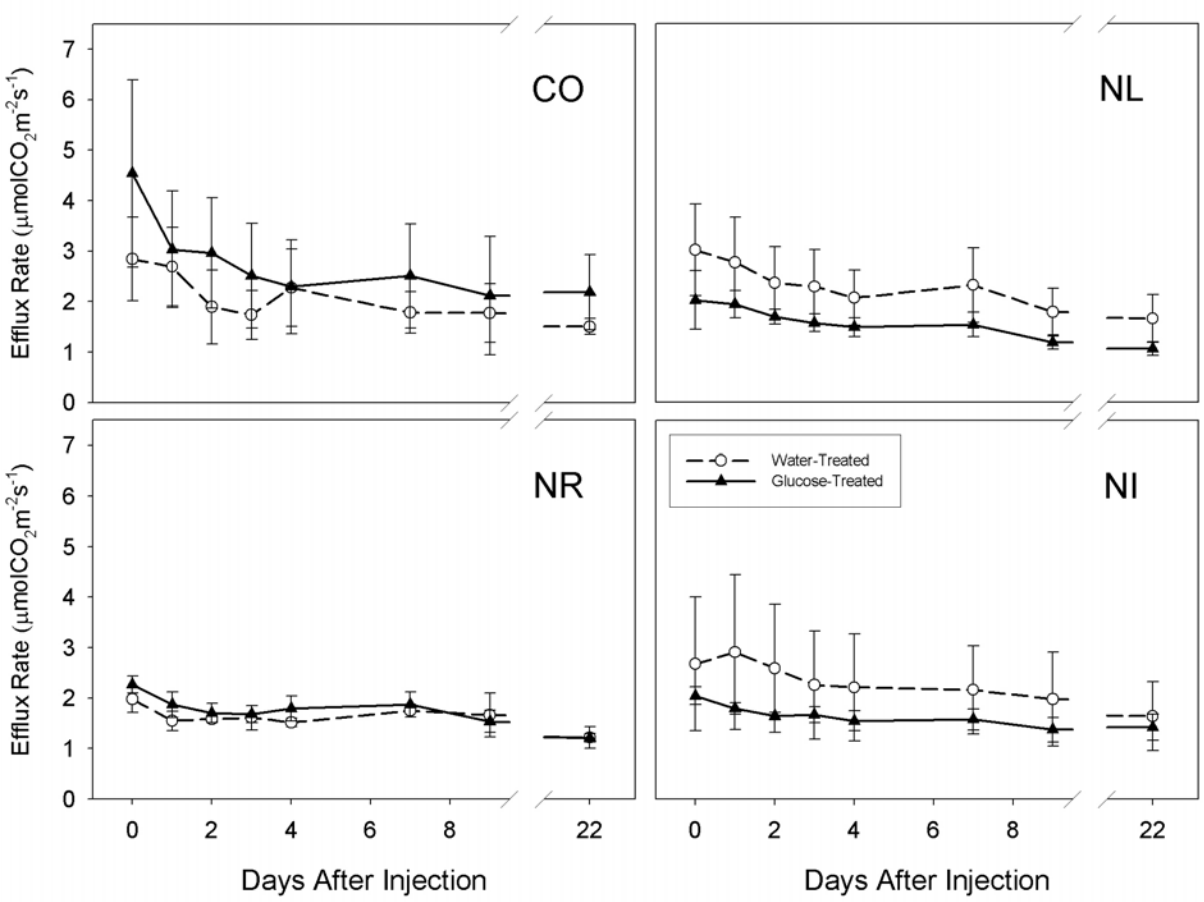
Notes: All points in this graph are single observations of calculated glucose-derived CO2 efflux rates (i.e., % glucose multiplied by the observed respiration rate). The area inside the dashed rectangle is zoomed in in the inset graph. In the larger graph, there is a break in the x-axis between days 9 and 22.

Figure 2.5: Priming Responses by DIRT Manipulation Type



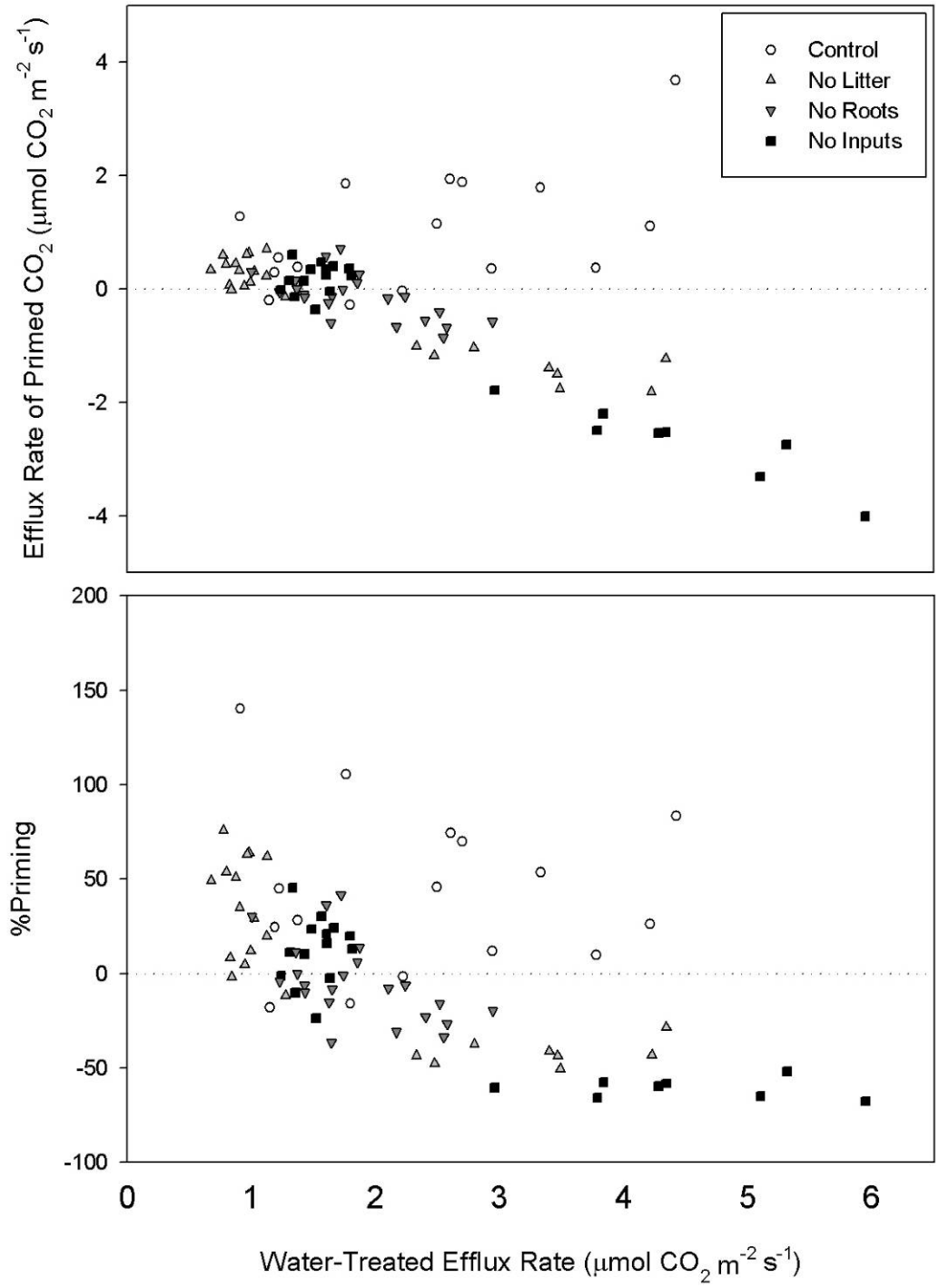
Notes: Error bars represent one standard error for all three DIRT replicate plots (n=3), except for Control treatment where n=2. Error bars are only shown for No Inputs (negative) and No Litter (positive) DIRT types to aid in visualization. The dashed grey line represents 0% change in SOM-derived CO_2 efflux between glucose and water treated collars. There is a break in the x-axis between days 13 and 19. Asterisks (*) indicate a priming value statistically significantly different from zero ($p \leq 0.05$).

Figure 2.6: SOM-Derived CO_2 Flux Rates



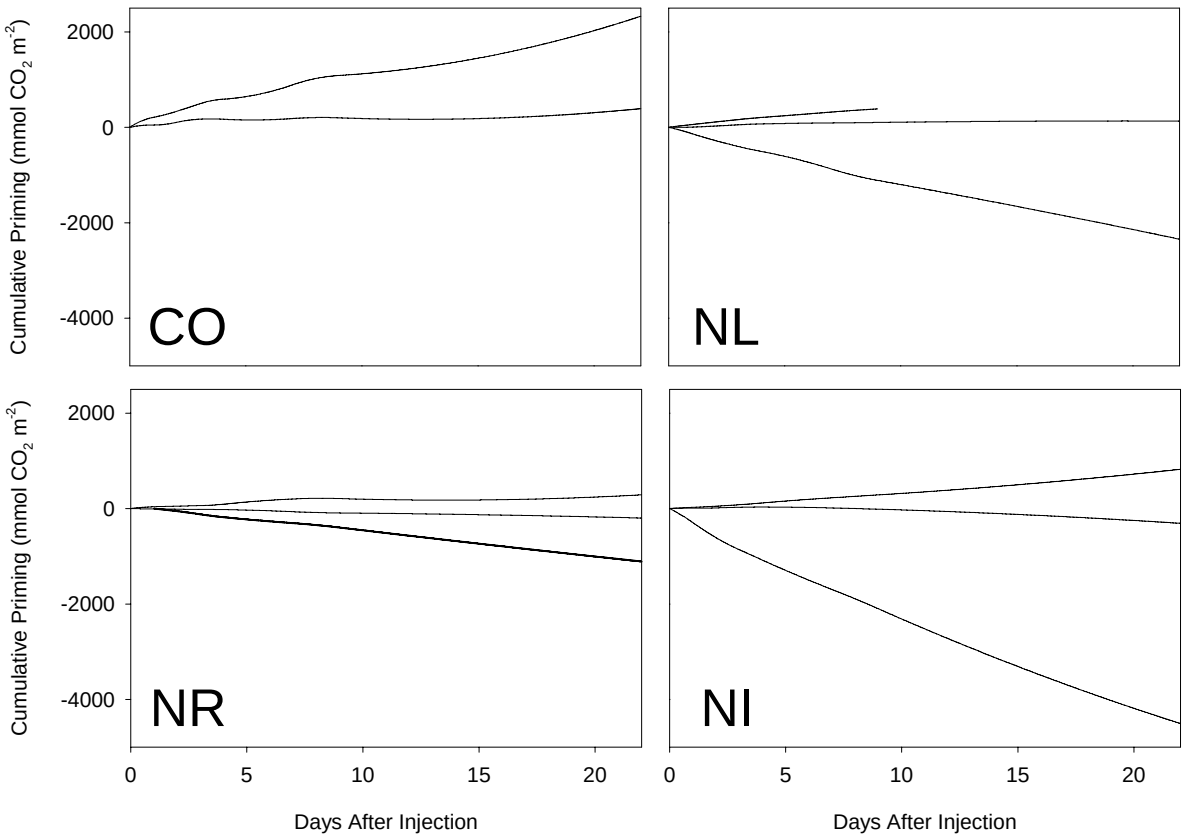
Notes: Graphs depict the average SOM-derived CO_2 flux rates for each DIRT manipulation. Two-letter codes correspond to Control (CO), No Litter (NL), No Roots (NR), and No Inputs (NI). All graphs have the same x and y-axis scales. Dotted lines correspond to the SOM-derived CO_2 flux from the glucose-treated collars while solid lines represent the total CO_2 flux from water-treated collars (all derived from SOM). The direction of priming is given by the relationship between these two lines, with positive priming corresponding to glucose-treated SOM-derived flux rates remaining higher than water-treated flux rates (as in the CO treatment) and negative priming corresponding to higher water-treated flux rates than glucose-treated SOM-derived flux rates. For all graphs there is a break in the x-axis between days 11 and 21.

Figure 2.7: %Priming and CO_2 Priming Rate in Relation to Background Respiration Rate



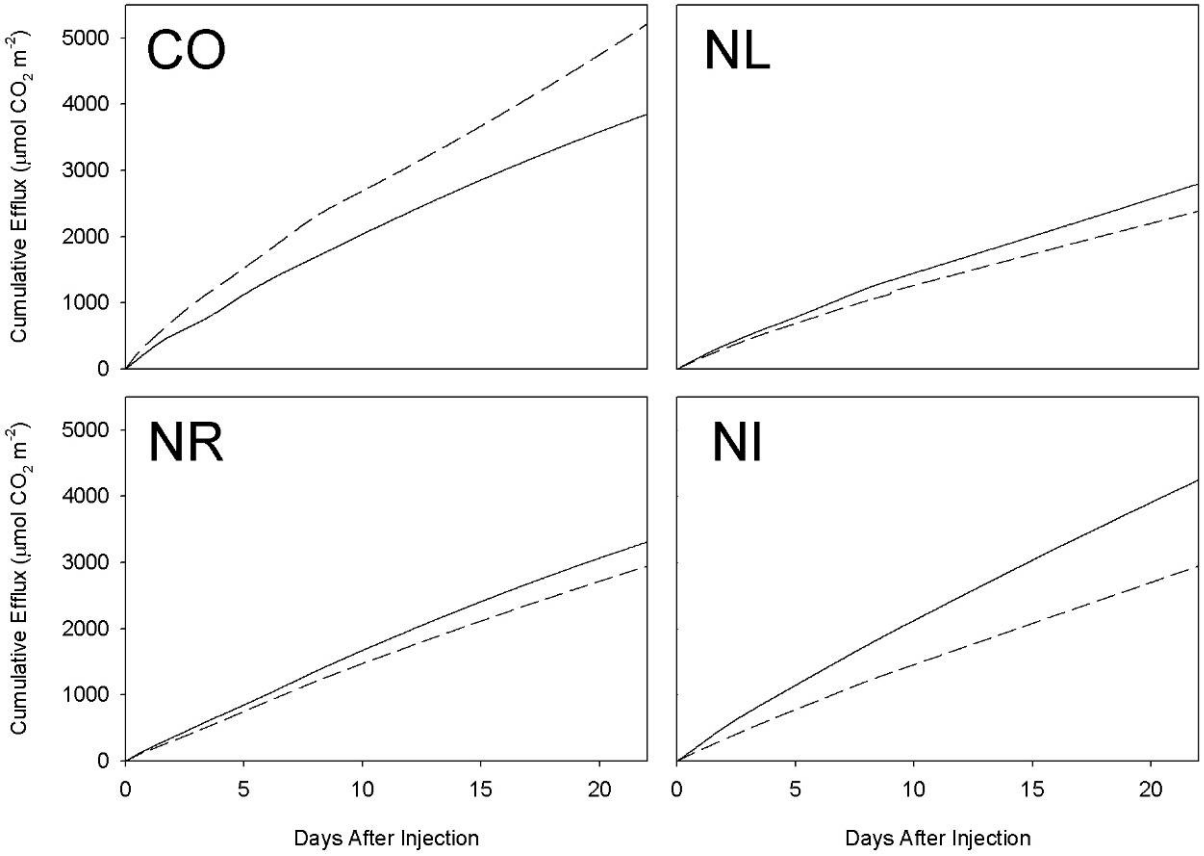
Notes: Thse graphs show the relationships between CO_2 priming measured as an efflux rate of primed CO_2 (upper graph) or as % priming measurements (lower graph) to the background respiration rate for each set of collars. Both graphs have the same x-axis scale and symbols.

Figure 2.8: Cumulative Priming for Each Collar



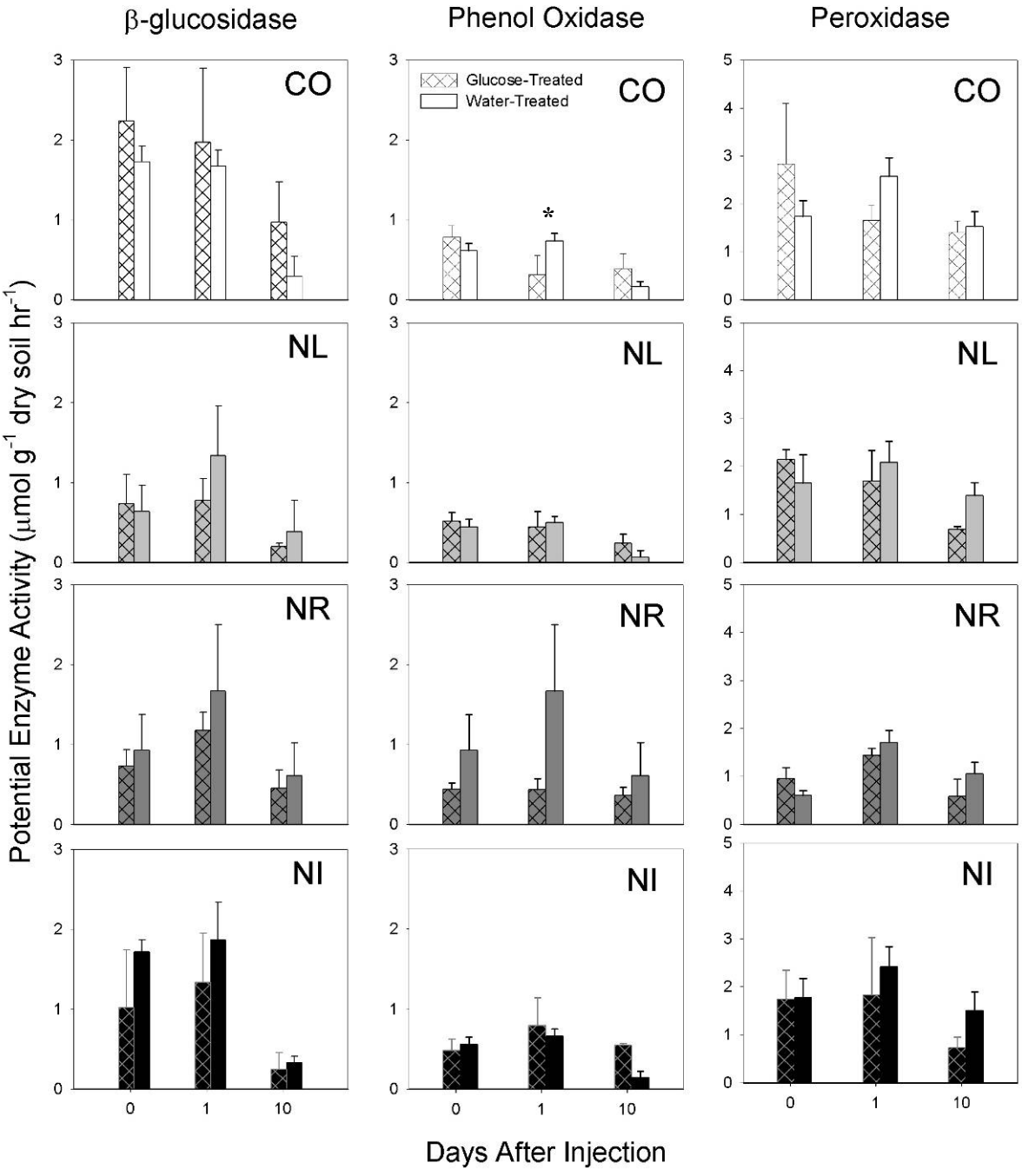
Notes: Each line represents the cumulative primed CO_2 for each collar where glucose was applied. All graphs have the same x and y-axis scales. Two-letter codes correspond to Control (CO), No Litter (NL), No Roots (NR), and No Inputs (NI). A priming estimate was unavailable for day 22 of one of the NL collars. No priming estimate was available for the first day of the lowest priming NR collar, so the cumulative priming value for that collar was begun at one day after injection.

Figure 2.9: Cumulative SOM-Derived CO_2 Flux



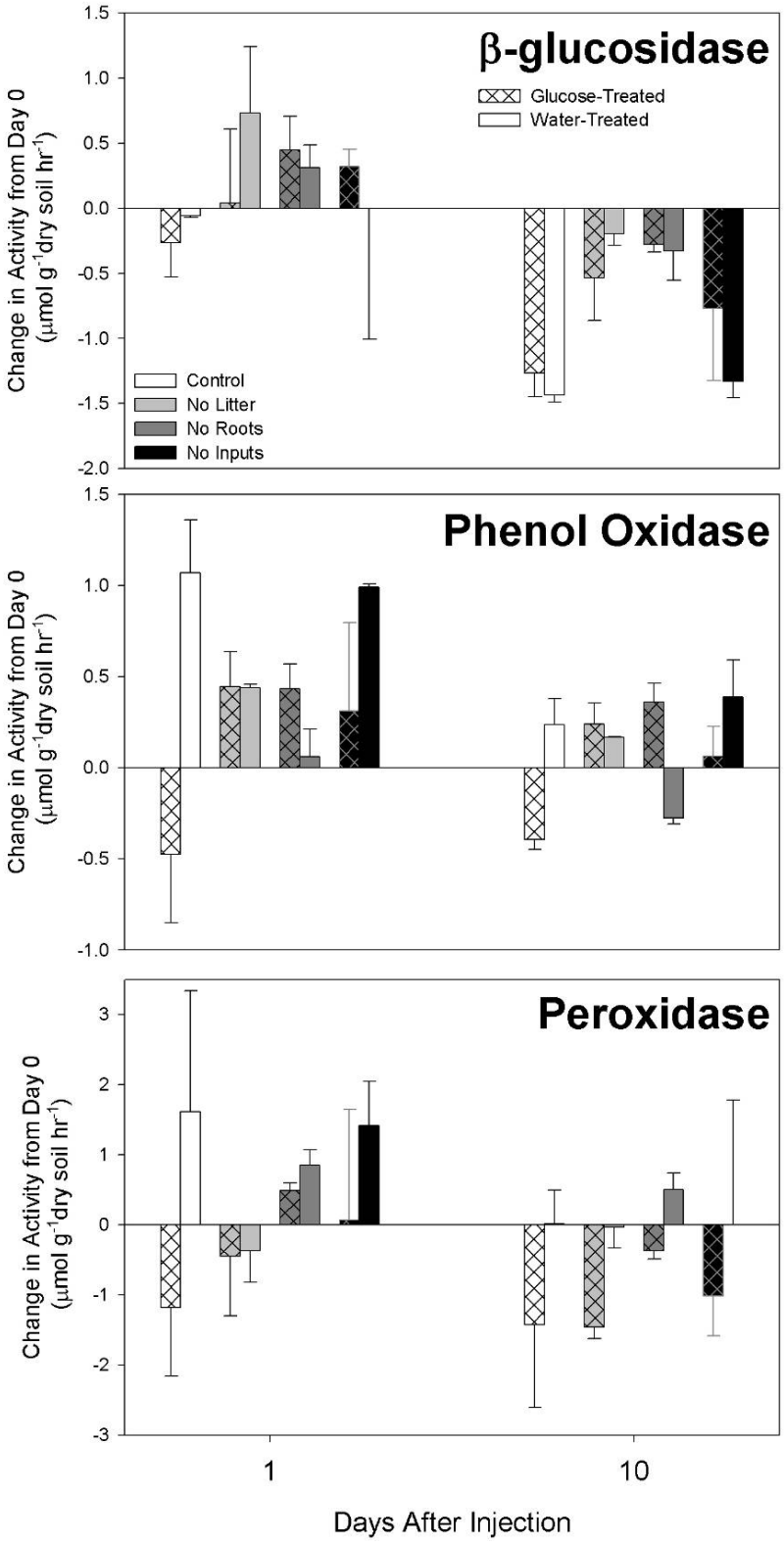
Notes: Graphs above depict the average cumulative values for each DIRT manipulation. Two-letter codes correspond to Control (CO), No Litter (NL), No Roots (NR), and No Inputs (NI). All graphs have the same x and y-axis scales. Dotted lines correspond to the SOM-derived CO_2 flux from the glucose-treated collars while solid lines represent the total CO_2 flux from water-treated collars (all derived from SOM). The direction of priming is given by the relationship between these two lines, with positive priming corresponding to glucose-treated SOM-derived flux remaining higher than water-treated flux (as in the CO treatment) and negative priming corresponding to higher water-treated cumulative flux than glucose-treated SOM-derived flux.

Figure 2.10: Enzyme Activities



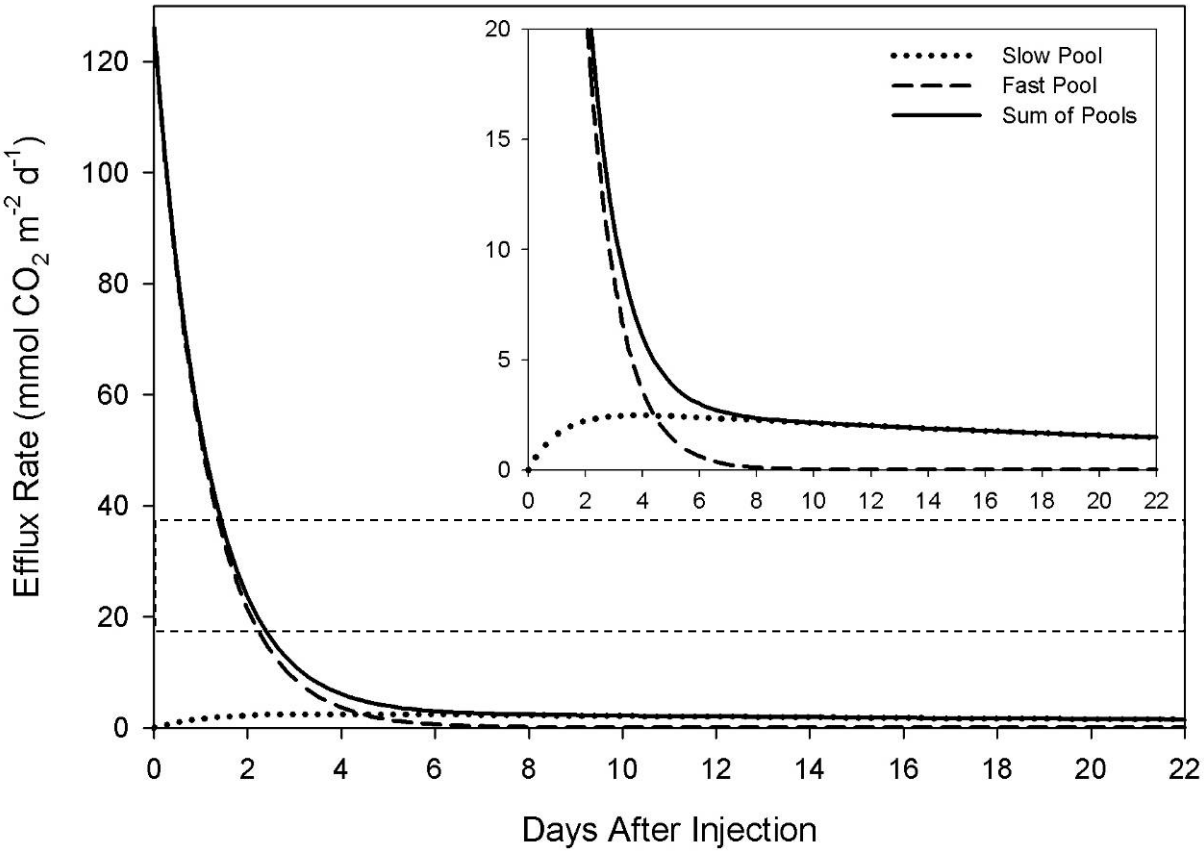
Notes: Each column of graphs corresponds to the enzyme labeled at the top of the column. DIRT manipulation types are inset within each graph – Control (CO), No Inputs (NI), No Litter (NL) and No Roots (NR). All graphs have the same units on the y axis (μmol of enzyme reaction product g^{-1} dry soil hr^{-1}) but not the same scale. Within each enzyme column, the y-axis scale is the same. Error bars represent one standard error for collars from all three DIRT replicate plots (n=3), except for Control water-treated where n=2. Asterisks (*) indicate a statistically significantly difference between water and glucose-treated collars zero (unadjusted $p \leq 0.05$).

Figure 2.11: Changes in Enzyme Activities



Notes: The y-axis presents the change in enzyme activities from those measured prior to injections on Day 0. Units for the y-axis represent μmol of enzyme reaction product g^{-1} dry soil hr^{-1}. Error bars represent one standard error for the three paired collars per DIRT manipulation type (*n*=3). All three graphs share the same x-axis as shown in the bottom graph, but do not have equal scales for the y-axis. Shades represent DIRT treatments (white=Control; light gray=No Litter; dark gray=No Roots; black=No Inputs). Hatching differentiates glucose-treated collars from water-treated collars.

Figure 2.12: Allocation in the Two Compartment Model



Notes: This graph shows both the overall model and the two individual compartments of the two-compartment model over time. Each line corresponds to the model-determined glucose-derived CO_2 flux rate coming from each pool over time. This model was fitted to observed values of glucose-derived CO_2 efflux rates for all plots. The area beneath the dashed line is shown zoomed in in the inset graph.

Table 2.1: Treatment Methods of the Detritus Input and Removal (DIRT) Plots.

Treatment	Method
Control	Normal litter inputs are allowed.
No Litter	Aboveground inputs are excluded from plots.
No Roots	Roots are excluded with impenetrable barriers extending from the soil surface to the top of the C horizon.
No Inputs	Aboveground inputs are prevented as in No Litter plots, belowground inputs are prevented as in No Roots plots.

Table 2.2: Enzyme Assay Reagents

Assay	Substrate	[Substrate]	Acetate Buffer	Stopper	Clay Flocculator
β-glucosidase	pNP-BG (Sigma N7006)	100 mM	100 mM (pH 5.5)	2 ml 0.1 M TRIS buffer (pH 12)	0.5 ml 0.5 M $CaCl_2$
Phenol oxidase	L-DOPA (Sigma D9628)	50 mM	50 mM (pH 5.0)	1 ml 0.6% Na azide	--
Peroxidase	L-DOPA (Sigma D9628) + 0.1 ml 0.3% H_2O_2	50 mM	50 mM (pH 5.0)	1 ml 0.6% Na azide	--

Notes: All enzyme substrates (Sigma-Aldrich, St. Louis, MO) were dissolved in the indicated buffer solution and 1 ml of substrate solution was added to each assay tube except for soil blanks. Substrate blanks received all reagents but no soil. Soil blanks received all reagents except enzyme substrate (i.e., acetate buffer without substrate).

Table 2.3: Cumulative Priming in Relation to C Pools and Fluxes

Collar	DIRT Treatment	Primed CO_2 (mmol CO_2 m^{-2})	% of MBC*	Net C Balance (mmol m^{-2})
21	CO	2330	68.6%	-1640
23	NI	823	70.3%	-141
15	CO	391	10.9%	+292
14*	NL	385	29.6%	+298
17	NR	287	12.4%	+396
24	NL	131	7.9%	+552
19	NR	-198	-10.7%	+881
18	NI	-309	-14.7%	+992
22*	NR	-1110	-77.0%	+1790
16	NL	-2340	-83.8%	+3030
20	NI	-4510	-270.9%	+5190

*Notes: Collar 14 values are based on 9-day cumulative values due to missing data on day 22. Collar 22 cumulative primed CO_2 was calculated beginning on day 1 rather than day 0 because no data was available for day 0. % of MBC was determined using a bulk density of 0.82 g cm^{-3} and the MBC value from day 0 of the glucose-treated collar. Net C Balance was calculated as the difference between the amount of C added as glucose and the amount given off through priming. The amount of injected glucose was 683 mmol C m^{-2}, so plots which showed negative priming as a result of glucose treatment gained the glucose C plus the amount of C not respired due to negative priming.

Table 2.4: Microbial Biomass Carbon (μg MBC g^{-1} dry soil)

Treatment	Day 0		Day 1		Day 10	
	Glucose	Water	Glucose	Water	Glucose	Water
Control	476.0 (±35.1)	426.0 (±119.6)	421.1 (±55.1)	292.0 (±62.7)	354.4 (±6.9)	231.2 (±14.9)
No Litter	280.6 (±66.3)	333.9 (±109.4)	267.0 (±72.1)	405.4 (±240.6)	237.7 (±68.3)	301.9 (±178.3)
No Roots	273.4 (±36.5)	316.9 (±73.4)	239.5 (±47.6)	276.0 (±37.2)	219.9 (±69.7)	234.0 (±48.7)
No Inputs	241.2 (±39.6)	254.5 (±20.7)	239.9 (±17.7)	240.7 (±12.0)	168.0 (±6.9)	270.9 (±64.4)

Notes: Values presented in parenthesis represent one standard error of collars per DIRT manipulation type (*n*=3), except for Control water-treated where *n*=2.

Table 2.5: Amount of Injected Glucose Found in Soil C Pools (%)

Treatment	Day 1			Day 10		
	MBC	**DOC**	**Insoluble***	**MBC**	**DOC**	**Insoluble***
Control	13.7 (±9.9)	2.1 (±1.5)	1.3 (±7.5)	22.5 (±8.3)	5.3 (±2.7)	18.7 (±13.9)
No Litter	6.7 (±0.3)	11.6 (±10.5)	14.4 (±12.1)	16.0 (±5.1)	3.6 (±1.7)	16.8 (±11.0)
No Roots	13.9 (±6.2)	7.1 (±3.3)	19.5 (±5.9)	14.2 (±4.6)	5.7 (±1.1)	30.5 (±2.3)
No Inputs	19.0 (±11.7)	7.6 (±4.8)	25.6 (±13.5)	20.7 (±1.3)	5.4 (±0.4)	27.7 (±5.1)

*Notes: Values in parenthesis represent one standard error for all three DIRT replicate plots (n=3), except for Control where n=2. Insoluble denotes C found in bulk soil that was not extracted after 1 hr treatment with 0.05M K_2SO_4, and was calculated by subtracting MBC and DOC from bulk soil measurements.

Table 2.6: Percentages of Added Glucose-C in Gas and Soil Pools

Pool	Day 1 (%)	Day 10 (%)
CO_2*	**12**	**23**
Bulk Soil	**37 (±10)**	**47 (±6.1)**
DOC	7.6 (±3.0)	5.0 (±0.7)
MBC	13 (±3.7)	18 (±2.2)
Insoluble	16 (±5.3)	24 (±3.9)
Missing	**51**	**30**
Total	**100**	**100**

*Notes: The amount of glucose-derived CO_2 may be underestimated by 20 to 30% based upon expected losses of glucose due to mineralization between injection and first efflux measurements. This estimate is based on the documented loss of 15% of glucose-C as CO_2 within one hour of amendment by Hill et al. (2008). Average percentages are shown for all DIRT plots considered ± one standard error (n=11). No standard error is shown for CO_2 because it was calculated by modeling the glucose-C flux using efflux data from all collars combined.

Table 2.7: Model Parameters for Glucose Decay Curves

DIRT Treatment	k_1 (d^{-1})	k_2 (d^{-1})	k_3 (d^{-1})	Yield Coefficient $k_2/(k_1+k_2)$
Control	0.534	0.372	0.026	41.1%
No Litter	0.538	0.336	0.028	38.4%
No Roots	0.392	0.235	0.026	37.4%
No Inputs	0.515	0.449	0.022	46.6%
Average	0.541	0.348	0.031	39.1%

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Chapter 3:
General Conclusions

David D. Diaz

Soils are a critical component of the global C cycle and changes in the rates of soil C turnover may be ecologically important in the midst of increasing CO_2 concentrations and climate change. The potential for positive feedbacks of soil respiration with elevated CO_2 makes the understanding of soil C turnover particularly important for predicting how natural ecosystems will respond to environmental changes. Though considerable effort has been focused on quantifying the fluxes and pool sizes of soil C in general, some of the fundamental mechanisms regarding the creation, persistence, and turnover of SOM are currently being reconsidered in light of recent studies utilizing new technologies. The acceleration of SOM turnover has been observed following the additions of organic substances through natural rhizodeposition, application of fertilizers and experimental substrates. Given that this priming of SOM turnover has been observed relating to both naturally occurring changes and common land-use practices, elucidating the mechanisms governing the priming effect would be a valuable addition to our understanding of how and why the largest biologically active terrestrial C pool may change over time.

This research project was designed to evaluate enzyme production as one of the most commonly cited mechanisms for the observed priming effect. By utilizing additions of glucose that were small relative to the amount of microbial biomass in soils, we aimed to isolate biomass growth responses from enzyme production. I was also interested in determining how different microbial communities relating to long-term manipulation of C inputs would respond to glucose additions. By modeling the efflux rates of CO_2 derived from SOM and from ^{13}C-labeled glucose, I was able to quantify the priming effects of glucose on SOM-turnover, evaluate the potential sources of this primed C within SOM and assess the turnover kinetics of glucose in soils. The key findings of this experiment were:

- There were no significant enzyme trends or patterns associated with glucose additions or observed priming effects, suggesting that the production of enzymes in response to availability of labile C is not the primary mechanism driving the priming of SOM.
- Glucose additions appeared to produce both preferential substrate utilization and more generalized suppression of respiratory activity in soils with restricted C inputs.

- Using relative measures of priming quantification which may be more sensitive to small but perhaps physiologically significant shifts in soils that show less background respiratory activity, the presence or absence of roots related directly to the to the direction of the priming effect.
- Absolute measures of primed C showed that all the soils with restricted C inputs generally responded with reduced turnover of SOM resulting in negative priming while soils without C input restrictions produced positive priming.
- Soils with higher background respiratory activity responded more negatively to glucose additions than did soils with lower background respiratory activities, but only in those soils which had received long-term restriction of C inputs. This relationship had not yet been documented in priming research.
- Comparing the cumulative amounts of primed CO_2 to the size of the MBC pool, it was not possible to exclude MBC as the sole source of primed C in all cases.
- The turnover of glucose-C in soils appeared to flow through at least two separate pools with turnover times of ~1d for the fast pool and ~30d for the slow pool.
- Yield efficiencies for glucose uptake into microbial biomass averaged ~40%, and are at the lower end of observed ranges for glucose uptake efficiencies.

Several of these findings support the working hypothesis of Fontaine et al. (Fontaine et al., 2003) that soil microbes do not produce enzymes in relation to substrate availability, and add to a growing body of research suggesting that a significant proportion of the C produced in SOM priming may derive from the turnover of intracellular microbial C reserves. Though I was unable to eliminate MBC as a sole source of primed C, I am skeptical that C-limited microbes in the bulk soil maintain intracellular reserves amounting to 50% or more of total MBC. The negative relationship between background respiration rates and priming response for both relative and absolute measures is a new finding in this field of research and suggests that the activities of microorganisms that have adapted to environments with restricted C inputs may be significantly disrupted following labile C additions, leading to net reductions in the turnover of SOM. Based on this relationship, it would be expected that active soil

microbial communities that have the bulk of metabolic activities performed by a specialized set of organisms may be most susceptible to changes in labile C inputs.

Future Research

Continued efforts should be focused on isolating microbial biomass growth responses from enzyme activities to determine whether soil microorganisms respond to changes in the availability of various substrates. Though considerable research has been focused on the priming effects of simple and complex C substrates, additions are often made at rates which greatly exceed natural input rates and may be many times larger than the size of the microbial biomass pool. Isolation of microbial biomass growth is a critical component in evaluating other mechanisms for priming effects, and experiments attempting to evaluate priming mechanisms should be done in such a way that C additions are within natural rates or that will produce negligible biomass growth.

The background variability of enzyme activities in soils makes experiments with increased replication potentially more valuable in determining the relationship between biomass growth, enzyme production, and substrate availability. Molecular characterization of microbial activities using methods such as quantitative polymerase chain reaction (qPCR) and measurements of ribosomal RNA (rRNA) may yield further insights into which groups of microorganisms respond to substrate additions and may be the most likely drivers of the priming effect. Tracing isotopically labeled C into phospholipid fatty acids and nucleic acids may also provide further insights into which organisms are incorporating the added C into microbial biomass.

The indirect documentation of priming at the DIRT plots relating to doubling of litter inputs by Sulzman et al. (2005) should also be further evaluated using more direct characterization of the priming effect following litter inputs and the composition and responses of microbial communities in those soils. Similarly, chemical characterization of the C environments and substrates available to microbial communities relating to DIRT C input manipulations using techniques such as pyrolysis and NMR may further elucidate how the microbial environment in soils has changed following altered C inputs. Regardless of the

mechanisms governing the priming effect, the potential for positive and negative C balances following C additions make priming an important aspect of C cycling and it should continue to be evaluated in more diverse soils and environmental conditions so that it may eventually be considered alongside other factors in models of soil carbon turnover and included in our evaluation of how soils may respond to altered CO_2 concentrations and global climate change.

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Appendix A: Soil Enzyme Assay Protocols

David D. Diaz

Slurry Preparation

The slurry created in this protocol was used to run 4 enzyme assays on each soil. One slurry for each soil sampled was split into several separate test tubes for enzyme assays.

1. Weigh 6 g of soil into 150 ml beaker.
2. Add 60 ml DI-H2O
3. Place on stirrer plate and mix vigorously with stirrer bar
4. During mixing, withdraw 1ml of slurry and put into all appropriate vials for enzyme assays (i.e., into soil blanks as well as soil +substrate duplicates). It is helpful to cut off the tip of the pipette tip to minimize clogging the hole with soil.
5. During mixing, withdraw 5 ml of slurry and place into pre-weighed tin boat, record which boat is used and weight of boat in data sheet
6. Place tin boat in drying oven at 105°C for 24 hours.
7. Weigh dried soil in tin boat to determine g of dry soil per ml of slurry.

β-glucosidase Protocol

Enzyme assays are run with duplicates for soils receiving substrate additions. One soil blank for each soil is made which does not receive substrate, but does receive CaCl and TRIS buffer solution. A substrate blank is also created without any soil, but receiving the substrate, CaCl, and TRIS buffer solution.

1. Add 1 ml of of soil slurry to each soil +substrate duplicate vials and to each soil blank vial. Do not add slurry to substrate blank.
2. Add 1ml 100mM acetate buffer to each soil blank.
3. Add 1ml pNP-BG substrate into all tubes except for soil blanks.
4. Vortex to mix.
5. Incubate in 30°C water bath for 1 hour.
6. While waiting on water bath, make a pNP standard curve
7. After water bath, add 0.5 ml of 0.5M $CaCl_2$ to all tubes.
8. Add 2 ml 0.1M TRIS Buffer to all tubes to stop reaction.
9. Vortex to mix.
10. Transfer to microcentrifuge tubes.
11. Centrifuge at 13,000 rpm (15,700 g) for 2 min.
12. Pipet 200 µL of supernatant from all tubes from beta-glucosidase assay into 96-well plate.
13. Measure absorbance in plate-reader at 410 nm.

Standard Curve for *p*-nitrophenol

1. To make the 5 µmol/ml pnp solution, put 3 ml stock solution (10 µmol/ml *p*-nitrophenol) into large test tube
2. add 3 ml DI water and vortex
3. Fill 9 tubes according to the chart below—use the 5 µmol/ml pNP solution

Tube #:	SA	SB	SC	SD	SE	SF	SG	SH	SI
100 mM Acetate (ml):	1	1	1	1	1	1	1	1	1
pNP (ml):	0	0.025	0.05	0.1	0.2	0.4	0.6	0.8	1
DI H_2O (ml):	1	0.975	0.95	0.9	0.8	0.6	0.4	0.2	0
µmols pNP in solution:	**0**	**0.125**	**0.25**	**0.5**	**1**	**2**	**3**	**4**	**5**

4. Add 0.5 ml 0.5 M $CaCl_2$ and 2 ml 0.1 M pH 12 Tris to all standard curve samples.
5. Vortex samples.
6. Transfer samples to microcentrifuge tubes and centrifuge at 13,000 rpm (15,700 g) for 2 min.
7. Pipet 200 µL of standard solutions from all tubes into 96-well plate for each assay.

Phenol Oxidase (Laccase) Protocol

Notes: No standard curve is used for this assay. Instead, a molar absorbance (extinction) coefficient of 3.7×10^{4} mol^{-1} L cm^{-1} was used.

1. Add 1 ml of of soil slurry to each soil+substrate duplicate vials and to each soil blank vial. Do not add slurry to substrate blank.
2. Add 1 ml 50mM acetate buffer to each soil blank.
3. Add 1 ml L-DOPA substrate into all tubes except for soil blanks.
4. Vortex to mix.
5. Incubate in 30°C water bath for 1 hour.
6. Add 1 ml of 0.6% Na azide (TOXIC = GLOVES) to all tubes to stop reaction.
7. Vortex to mix.
8. Transfer to microcentrifuge tubes.
9. Centrifuge at 13,000 rpm (15,700 g) for 2 min.
10. Pipet 200 µL of supernatant from all tubes from beta-glucosidase assay into 96-well plate.
11. Measure absorbance in plate-reader at 460 nm.

Peroxidase Protocol

Notes: No standard curve is used for this assay. Instead, a molar absorbance (extinction) coefficient of 3.7×10^{4} mol^{-1} L cm^{-1} was used. This essay is identical to the phenol oxidase assay with the exception of adding H_2O_2 in step 4.

1. Add 1 ml of of soil slurry to each soil+substrate duplicate vials and to each soil blank vial. Do not add slurry to substrate blank.
2. Add 1 ml 50 mM acetate buffer to each soil blank.
3. Add 1 ml L-DOPA substrate into all tubes except for soil blanks.
4. Add 0.1 ml of 0.3% Hydrogen Peroxide to all tubes.
5. Vortex to mix.
6. Incubate in 30°C water bath for 1 hour.
7. Add 1 ml of 0.6% Na azide (TOXIC = GLOVES) to all tubes to stop reaction.
8. Vortex to mix.
9. Transfer to microcentrifuge tubes.
10. Centrifuge at 13,000 rpm (15,700 g) for 2 min.
11. Pipet 200 µL of supernatant from all tubes from beta-glucosidase assay into 96-well plate.
12. Measure absorbance in plate-reader at 460 nm.

Appendix B: Statistical Output from RM-ANOVA

David D. Diaz

Respiration Rates:

Dependent Variable	resp
Covariance Structure	Autoregressive
Subject Effect	plot(label)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	plot(label)	0.9457
Residual		1.6886

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
DIRT	3	15.2	0.69	0.5726
day	7	104	19.19	<.0001
DIRT*day	21	103	1.42	0.1271
label	1	15.2	0.11	0.7451
DIRT*label	3	15.2	0.22	0.8840
label*day	7	104	9.08	<.0001
DIRT*label*day	21	103	2.01	0.0111

% Priming:

Dependent Variable	priming
Covariance Structure	Autoregressive
Subject Effect	plot
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	plot	0.8450
Residual		0.1673

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
DIRT	3	7.92	1.29	0.3422
day	7	42.7	4.09	0.0016
DIRT*day	21	40.7	3.39	0.0004

Moisture Content:

Dependent Variable	Moisture
Covariance Structure	Autoregressive
Subject Effect	plot(Label)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	plot(Label)	0.8573
Residual		0.001218

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
DIRT	3	15.1	1.11	0.3758
Day	2	29.7	22.19	<.0001
DIRT*Day	6	29.7	1.03	0.4232
Label	1	15.1	0.35	0.5622
DIRT*Label	3	15.1	0.73	0.5516
Label*Day	2	29.7	0.92	0.4083
DIRT*Label*Day	6	29.7	0.84	0.5459

Microbial Biomass Carbon:

Dependent Variable	Biomass
Covariance Structure	Autoregressive
Subject Effect	plot(Label)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	plot(Label)	0.7432
Residual		0.01860

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
DIRT	3	17.1	1.22	0.3340
Day	2	31.3	3.55	0.0409
DIRT*Day	6	31.3	0.88	0.5217
Label	1	17.1	0.07	0.7888
DIRT*Label	3	17.1	0.57	0.6415
Label*Day	2	31.3	0.00	0.9952
DIRT*Label*Day	6	31.3	0.67	0.6713

β-glucosidase Activities:

Dependent Variable	Bgase
Covariance Structure	Autoregressive
Subject Effect	plot(Label)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	plot(Label)	0.6451
Residual		0.5805

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
DIRT	3	18.4	1.47	0.2556
Day	2	32.2	28.54	<.0001
DIRT*Day	6	32.4	1.20	0.3323
Label	1	18.4	0.17	0.6854
DIRT*Label	3	18.4	0.53	0.6655
Label*Day	2	32.2	1.18	0.3195
DIRT*Label*Day	6	32.4	0.22	0.9675

Phenol Oxidase (Laccase) Activities:

Dependent Variable	Laccase
Covariance Structure	Autoregressive
Subject Effect	plot(Label)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	plot(Label)	-0.2577
Residual		0.06882

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
DIRT	3	29.1	8.22	0.0004
Day	2	32.3	11.75	0.0001
DIRT*Day	6	33.6	0.93	0.4896
Label	1	29.1	0.63	0.4327
DIRT*Label	3	29.1	1.99	0.1370
Label*Day	2	32.3	4.11	0.0256
DIRT*Label*Day	6	33.6	0.64	0.6968

Peroxidase Activities:

Dependent Variable	Perox
Covariance Structure	Autoregressive
Subject Effect	plot(Label)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	plot(Label)	0.1368
Residual		1.2003

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
DIRT	3	19.6	2.49	0.0899
Day	2	31.5	4.83	0.0148
DIRT*Day	6	32.2	0.49	0.8119
Label	1	19.6	2.85	0.1073
DIRT*Label	3	19.6	0.44	0.7242
Label*Day	2	31.5	1.88	0.1696
DIRT*Label*Day	6	32.2	0.43	0.8534