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Root herbivores accelerate carbon inputs to soil and drive changes in biogeochemical processes

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ABSTRACT

Root herbivory is a pervasive rhizosphere process but its role in regulating root inputs to soil and subsequent impact on soil organic matter has been largely overlooked. We present the first manipulative field study investigating the effects of root-feeding Japanese beetle larvae on soil organic matter cycling under tall fescue. Our results show that root-feeding larvae cause an increase of plant photosynthetic products in soil over a 7-month study period. However, soils exposed to root herbivores also exhibited a ~ 8% decrease in total soil carbon, along with a 13% increase in microbial biomass carbon, and a 16% increase in microbial biomass nitrogen. In addition, there was a marginally significant increase in microbial extracellular glucosidase and cellobiohydrolase activities and a decrease in oxidase activities. These findings highlight the potential of root herbivores to accelerate root inputs to soil and stimulate the decomposition of existing soil organic matter.

There is growing evidence that plant roots and root-derived products play a central role in the formation and breakdown of soil organic matter (SOM) (Jackson et al., 2017; Rasse et al., 2005). Biological interactions in the rhizosphere, e.g., mycorrhizal symbiosis, can regulate the transfer of root products to different soil organic carbon pools (Averill et al., 2014; Brzostek et al., 2015). Root herbivory by diverse taxa of insects, nematodes, and rodents is another pervasive rhizosphere interaction in most terrestrial ecosystems (Andersen, 1987). By consuming root tissues and introducing waste products to soil, root herbivores presumably present another important biotic control on material flow from plants to soil (Hunter, 2001). Indeed, a few lab studies have revealed an increase in rhizodeposition upon infection by root-parasitic nematodes (Haase et al., 2007) and a surge in dissolved organic matter in response to root chewing insects (Treonis et al., 2005). Root herbivores have also been shown to increase microbial utilization of labile organic substrates and to alter microbial community composition in pot studies (Dawson et al., 2004; Wurst and van der Putten, 2007). However, it is unclear whether these patterns observed in controlled lab/greenhouse settings would apply under field conditions. In addition, it remains unknown whether herbivore-induced changes in soil microbial communities can have any significant impact on SOM dynamics under realistic field environments.

Here we present the first field study investigating the consequences of belowground insect herbivores for plant inputs to soil and their subsequent impacts on soil carbon (C) cycling. We first hypothesized that root herbivores would increase the introduction of plant materials into soil. Second, we hypothesized that herbivore-induced inputs would alter the structure and function of the soil decomposer community, resulting in further changes to SOM pools.

We focused our study on the root feeding larvae of the Japanese beetle (*Popillia japonica* Newman) (Coleoptera: Scarabaeidae), which was introduced to North America over 100 years ago and is now widespread in the eastern USA. Adult Japanese beetles are notorious foliar feeders on over 300 plant species and the larvae feed primarily on roots of the Poaceae (Potter and Held, 2002). The study was conducted within a 3 m x 5 m stand of grass, dominated by tall fescue (*Festuca arundinacea* Schreb) and free of *P. japonica* larvae, enriched with ^{13}C-CO_2 (152.0 $\delta^{13}C$ in grass shoots, supplement Text S1). Following the completion of the enrichment, five one m^{-2} blocks were created within the enriched area, and each block received 3 polyvinyl chloride (pvc) collars (15 cm in diameter, 12 cm in depth) for a total of 15 collars. The collars were inserted fully into the soil on Oct 16th 2015 to serve as containment feeding arenas. The three arenas within each block received one of three density treatments of third-instar *P. japonica* larvae: larva-free control, moderate, and high density with 0, 3 and 6 larvae added respectively inside each pvc arena. “Moderate” (165 individuals m^{-2}) represented a larval density just high enough to cause economic damage (Crutchfield and Potter, 1995), while “high” (330 individuals m^{-2}) represented a heavy outbreak density of *P. japonica* (Fleming, 1972).

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The pvc arenas were harvested on May 9th of 2016 before the larvae began to pupate, permitting two separate feeding periods for *P. japonica* (fall of 2015 and spring of 2016). Three soil cores (2 cm in diameter, 10 cm in depth) were collected within each arena for arthropod extraction before the pvc rings were excavated with a shovel. Soil columns removed from each arena were sorted gently to remove any root-feeding insect larvae and all live plants were removed. One quarter of the homogenized soil (~500 g) and the separated plant biomass therein (both shoots and roots) was bagged separately and transported to the lab. In the lab, we quantified (1) traits related to plant inputs including root biomass, root to shoot ratio, and new photosynthates in soil (calculated as ^{13}C excess in the experimental plots compared to a nearby unlabeled area of the same grass community); (2) biotic factors involved in the decomposition process including microbial biomass C and biomass nitrogen (N), C and N-degrading extracellular enzyme activities, and the abundance of decomposer microarthropods (i.e., collembolans and mites) other than root feeding larvae; and (3) SOM pools including total C and N content, permanganate oxidizable C, and amino-sugar biomarkers (muramic acid, glucosamine, and galactosamine) for microbial necromass (Amelung, 2001). Protocols for each measurement can be found in supplement Text S1.

Larval survival was assessed and confirmed by harvesting one of our arenas one month following the start of the experiment, however, live *P. japonica* were present in only 4 out of the 10 arenas originally receiving larvae and no distinctions remained among the initial "moderate" and "high" larval treatments at the end of the 7-month study period (Table S2). As a result, we merged our two density treatments into a single treatment (*P. japonica* added). We conducted linear mixed models for each soil trait using herbivore treatment (control vs. *P. japonica* added) as a fixed factor and block as a random effect. We also performed a second modeling using the presence/absence of live *P. japonica* larvae at harvest to further test any effect due to the continued presence of live larvae throughout the study. Another species of root-feeding larva (*Maladera castanea* Arrow) was also present at harvest in 3 control arenas and 2 *P. japonica* arenas (Table S2). While their densities were below economic damage levels for this species, we chose to include them as a random factor in the mixed model to remove any potential confounding effects. All data analyses were performed using R software (R Core Team, 2016), with *nlme* for linear mixed model analysis (Pinheiro et al., 2018), and *lsmeans* for subsequent Tukey's *post hoc* multiple comparisons (Lenth, 2016).

Soils exposed to *P. japonica* during the study period had lower total soil C (Fig. 1a) and soil N than control soils (Fig. 1b), although there was no difference in the permanganate oxidizable C or microbial necromass (Table S1). Two additional studies have observed a negative correlation between root herbivore density and SOM content (Dimock 2004; Madsen 2009), and the data from Madsen (2009) reveal a similar difference in SOM (~8%) between soils with and without root herbivores. The magnitude of decline in SOM in response to root herbivory is comparable to what has been observed in response to aboveground herbivory by mammalian grazers (up to 10% reduction in SOC; McSherry and Ritchie, 2013; Zhou et al., 2017).

The reduction in SOC under root herbivory may have been driven by two primary factors: first, root removal can reduce overall plant productivity and C allocation to roots (Zvereva and Kozlov, 2012), which will eventually reduce the total C input to soil from senesced root and leaf litter. In our study, arenas receiving *P. japonica* tended to have lower root biomass and lower root to shoot ratio than *P. japonica*-free controls, but the differences were not significant (Table S1). Alternatively, the lower soil C content observed in the *P. japonica*-exposed treatment could be due to herbivore-induced "priming" of existing SOM (Blagodatskaya and Kuzyakov 2008). Herbivore-induced inputs, either feces, rhizodeposition, or even cadavers are likely to contain more labile compounds than the otherwise senesced root litter. Labile inputs can stimulate microbial decomposition of not only the new input but also the existing SOM, resulting in additional CO_2 respired relative to what would be respired from the input alone ("real priming" as defined in Blagodatskaya and Kuzyakov 2008). As increases in microbial biomass and extracellular enzyme activities are two main indicators of real priming (Blagodatskaya and Kuzyakov 2008), our study partly supported the "priming" hypothesis in that soil receiving *P. japonica* had a higher amount of microbial biomass C (Fig. 1d), and microbial biomass N (Fig. 1e). The increase in microbial biomass may have "cascaded up" to benefit decomposer arthropods, indicated by an increase in the density of oribatid mites (Fig. 1f), many of which are microbivores (Schneider et al., 2004). The *P. japonica* treatment also had a marginal stimulatory effect on C-degrading enzymes (glucosidase, Fig. 1g; cellulase, Fig. 1h). However, root herbivory appeared to suppress phenol oxidase activity (Fig. 1i). As fungi are the primary producer of oxidative enzymes in soil (Sinsabaugh, 2010), the contrasting response of hydrolytic and oxidative enzymes may suggest a decline in fungal biomass and fungal: bacterial ratio under root herbivory similar to that observed in aboveground grazing studies (Bardgett et al., 2001; Klumpp et al., 2009).

Laboratory studies on root-feeding nematodes have also found that herbivory increases the flow of plant-derived inputs to soil microorganisms and increased microbial biomass (Bardgett et al. 1999; Yeates et al., 1999). In our study, the herbivore treatment appeared to have little impact on the amount of new ^{13}C in soil (Fig. 1c, Table S1). However, when data were analyzed based on the presence or absence of live *P. japonica* upon harvest, soil ^{13}C content was significantly greater in soils containing live, presumably actively feeding, *P. japonica* (Fig. 2). This increase in ^{13}C input under active feeding may have been due to the continuous release of soluble materials from roots ("leakage") under root feeding (Haase et al., 2007; Treonis et al., 2007), and the conversion of root materials to feces upon gut passage (Potter et al., 1992). The disappearance of excess ^{13}C in soil lacking live *P. japonica* (Fig. 2) suggests that these herbivore-induced inputs are likely to be utilized by microbes quickly and may have contributed to "priming" effects as discussed above. No other measurements showed statistical differences when analyzed based on the presence or absence of live *P. japonica* upon harvest (data not shown).

In addition to changes in SOC pools, we also observed a decline in soil N content in arenas receiving *P. japonica* (Fig. 1b). As insect feces are known to contain high N particularly in the form of ammonium (Kagata and Ohgushi 2012), and given the fact that the N uptake ability of the plants is compromised by root herbivory, we posit that the decline in soil N may be due partly to the potential leaching of N, although the restriction of our measurements to the top 10 cm of soil prevented us from testing this in the present study.

We remain cautious in interpreting our results due to our inability to track fully the intensity and duration of herbivory within each arena throughout the study. Despite this caveat, which is common to many field mesocosm studies, our findings suggest that, under field conditions, root chewing insects can increase the transfer of photosynthetic products in soil, which may stimulate the decomposer community and result in an overall decline in total C and N in soils. Whether the patterns observed here occur in other belowground plant-insect systems remains to be determined, however the potential for root herbivores to induce priming-like effects on soil microbial communities warrants a better understanding of the role of herbivores in SOM cycling. This knowledge will be crucial in improving our predictions of SOM responses to environmental changes, especially in ecosystems where root herbivores are undergoing large shifts in population dynamics, either due to their sensitivity to climate change (Hiltpold et al., 2017) or the introduction of root herbivores into new regions (Miller et al., 2005).

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