# PREDATOR CONTRIBUTIONS TO BELOWGROUND RESPONSES TO WARMING

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# ABSTRACT

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Identifying the factors that control soil carbon dioxide ($CO_2$) emissions will improve our ability to predict the magnitude of climate change-soil ecosystem feedbacks. Despite the integral role of invertebrates in belowground systems, they are excluded from climate change models. Soil invertebrates have consumptive and non-consumptive effects on microbes, whose respiration accounts for nearly half of soil $CO_2$ emissions. By altering the behavior and abundance of invertebrates that interact with microbes, invertebrate predators may have indirect effects on soil respiration. This research examined the effects of a generalist arthropod predator on belowground respiration under different warming scenarios. Based on research suggesting invertebrates may mediate soil $CO_2$ emission responses to warming, predator presence was predicted to result in increased emissions by negatively affecting these invertebrates. Presence of the predator, wolf spiders (*Pardosa spp*.), was manipulated in mesocosms containing a community of soil invertebrates. To simulate warming, we placed mesocosms of each treatment in ten open-top warming chambers ranging from 1.5 to 5.5° C above ambient at Harvard Forest, MA. Soil $CO_2$ efflux data, microbial abundance, soil moisture, and soil temperature were measured to determine the effects of predators on belowground systems. As expected, $CO_2$ emissions increased under warming and there was an interactive effect of predator presence and warming, though the effect was not consistent through time. The interaction between predator presence and temperature was the inverse of our predictions: mesocosms with predators had lower $CO_2$ emissions at higher temperatures than those without predators. $CO_2$ emissions were not significantly associated with microbial biomass or soil moisture. There was not find evidence

of consumptive effects of predators on the invertebrate community, suggesting that predator presence mediates response of microbial respiration to warming through non-consumptive means. In this system we found a significant interaction between warming and predator presence that warrants further research into mechanism and generality of this pattern to other systems.

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# INTRODUCTION

Understanding what controls the rate of $CO_2$ release from the soil is imperative. Soil carbon emissions account for 10x more $CO_2$ release than anthropogenic inputs, and the potential for positive climate-soil carbon emission feedbacks makes identifying the mechanisms behind soil carbon emissions important (IPCC, 2013; Schlesinger & Andrews, 2000). Currently, Earth System Models (ESMs) used to predict climate change include only microbial and plant respiration in calculations of soil $CO_2$ emissions (Wieder, Bonan, & Allison, 2013). This approach fails to take into account the complexity of soil food webs, which are comprised largely of invertebrates that control microbe and plant dynamics. Soil invertebrates are left out of ESMs because their direct contribution to soil respiration is trivial relative to that of plant roots (~45% of total soil respiration) and microbes (~55% of total soil respiration) (Hanson, Edwards, Garten, & Andrews, 2000; Lavelle, 1997; Schlesinger & Andrews, 2000); however, invertebrates have significant indirect effects on soil carbon release, particularly through their role in the detrital food web.

Soil invertebrates can directly and indirectly alter soil microbe abundance and community structure. By consuming microbes, invertebrates directly alter their abundance (reviewed in David A. Wardle, 2002). Often, invertebrates feed selectively, altering microbe community structure (Crowther, Jones, & Boddy, 2011; Ronn, McCaig, Griffiths, & Prosser, 2002; D. A. Wardle, 2006), which may be important in determining soil $CO_2$ emissions (De Vries et al., 2013; Strickland, Lauber, Fierer, & Bradford, 2009). Strickland et al. (2009) suggest that microbe community structure may explain up to 20% variation of total carbon respiration.

Invertebrates affect the microbial community indirectly as well. Invertebrates such as earthworms, ants, and millipedes act as 'ecosystem engineers', physically altering the soil environment through means such as burrowing, shredding detritus, and excreting labile carbon and nutrient sources, which are readily broken down by microbes (Del Toro, Ribbons, & Ellison, 2015; Lavelle, 1997; Nielson, Ayres, Wall, & Bardgett, 2011; D. A. Wardle, 2006). Since soil microbes are restricted in their ability to move to new resources and they depend partly on invertebrates to provide new substrates, the activity of these ecosystem engineers stimulates microbes (Lavelle, 1997; Rantalainen et al., 2004). In fact, the presence of invertebrates has been shown to contribute to increased soil respiration (De Vries et al., 2013; Del Toro et al., 2015; Fox, Vetter, Ekschmitt, & Wolters, 2006; Lavelle, 1997; Pelini, Maran, Chen, Kaseman, & Crowther, In Review) and decomposition (Wall, Bradford, St. John, & et.al, 2008), particularly in systems not constrained by temperature or precipitation (e.g. temperate and wet tropical biomes).

The responses of soil invertebrates to warming are varied. A meta-analysis by Blankinship, Niklaus, and Hungate (2011) suggests that differences between studies may be explained by the climate where the study is conducted and from which the study organisms originated. In colder and drier climates, Blankenship et al. found stronger negative effects on invertebrate abundances, and these effects were more likely to be observed in long-term experiments. This meta-analysis focused on changes in abundance, but not community structure, to warming. Warming has been shown to change invertebrate community structure (Bokhorst, Huiskes, Convey, van Bodegom, & Aerts, 2008; Briones, Ostle, McNamara, & Poskitt, 2009; Pelini et al., 2014; Zhang, Li, Lu, Zhang, & Liang, 2013), and as with microbes, a change in invertebrate community structure may alter ecosystem functioning (Heemsbergen et al., 2004).

Changes in invertebrate and/or microbial community structure are particularly important when they result in reductions in functional diversity, as more variation in functional roles can lead to facilitative interactions that promote decomposition (Heemsbergen et al., 2004). In addition, the presence of invertebrates that selectively graze on fungi has been shown to prevent warming effects on fungi (A'Bear, Boddy, & Jones, 2013) suggesting that community structure could determine the role of invertebrates in mediating ecosystem responses to warming.

Invertebrate community structure may be altered by generalist predators of soil invertebrates, such as wolf spiders. Such changes may impact soil respiration under warming considering the importance of invertebrates for ecosystem functioning and the potential for warming to disrupt their activity. By consuming detritivores and microbivores, predators exert top-down control on microbes (Gessner et al., 2010), and therefore have cascading effects on soil respiration. Further, by selectively grazing, predators may alter invertebrate community structure (Gessner et al., 2010). However, indirect effects of predator presence on invertebrate behavior have been shown to be equal to or greater than direct consumptive effects (Preisser, Bolnick, & Benard, 2005; Strickland, Hawlena, Reese, Bradford, & Schmitz, 2013). There is evidence of predator presence having far reaching effects, including on rates of decomposition (Hawlena, Strickland, Bradford, & Schmitz, 2012), soil respiration (Sitvarin & Rypstra, 2014), and plant biomass (Zhao, Griffin, Wu, & Sun, 2013). These indirect effects of predators may be even more important under climate change since warming has been shown to amplify the top-down effects of predators (Barton, Beckerman, & Schmitz, 2009; Jochum, Schneider, Crowe, Brose, & O'Gorman, 2012).

To summarize, although invertebrate predators play pivotal roles in soil carbon cycling, they are rarely included in empirical inquiries and models used to determine if soil $CO_2$

emissions, via respiration of soil fauna, will increase under warming, generating a positive carbon-climate change feedback. Invertebrates are missing from these efforts because their collective respiration is presumed to be small relative to that of microbes. However, the indirect effects of invertebrates on soil respiration through interactions with plant and microbial communities are significant and will be altered by warming. Because invertebrates are ectotherms, warming alters their rates of predation (Chown & Nicolson, 2004), thereby altering the soil food web and consequently microbial and plant respiration. The objective of this study was to determine the contributions of soil invertebrate predators to soil respiration under different warming scenarios. This study focused on the detrital part of the belowground food web (Figure 1). My central hypothesis is that predator presence will amplify the increase of soil respiration under warming because:

1. The predators will be more active at higher temperatures, i.e., consuming microbivores at a higher rate, thereby increasing microbe abundance, resulting in higher respiration belowground.
2. The presence of a predator will cause more burrowing and fear response from the belowground invertebrates, which will provide more substrate for the microbes and result in higher respiration belowground in higher temperatures.

To test the hypothesis, the presence of wolf spiders (*Pardosa spp.*) was altered in mesocosms containing soil fauna. Warming was simulated by placing mesocosms of each treatment in ten open-top warming chambers ranging from 1.5-5.5°C above ambient at Harvard Forest, MA.

# METHODS

## Study Site

Mesocosms were placed in 5 meter diameter, open-top warming chambers at Harvard Forest, Petersham, MA. The chambers have been running continuously for five years. The temperature inside each chamber is raised a set level above the ambient temperature via a forced air system. The levels are at .5°C increments from 1.5-5.5°C above ambient, which resulted in 10 warming treatments (Figure 2). For a detailed explanation of how the heating system works, see Pelini et al. (2011). The chambers are located in a 70-year-old oak-maple stand in the Prospect Hill Tract (Pelini, et al. 2011).

## Invertebrates

Springtails (Collembola), mites (Acari), and millipedes (Diplopoda) were extracted from litter collected within 100 m of the warming chambers. The invertebrates were separated from the litter by hand sieving first with 2 mm, followed by 1 mm mesh sieves. This method was used both to collect the invertebrates and to determine natural densities of each taxa in the study area. Adult male wolf spiders (*Pardosa spp.*) and earthworms (Lumbricidae) were collected by hand in an overgrown vegetable garden within 100 m from the study site. Care was taken to select individuals of similar sizes in macroscopic taxa: millipedes were approximately 1 cm in length and worms were approximately 4.5 cm in length. Feeding trials were conducted to verify that the wolf spiders collected would eat potential prey in the mesocosm (i.e. springtails, millipedes, earthworms), as found in a recent isotopic study (Wimp et al., 2013). The trials were done three

times for each prey item and the spider consumed all prey offered, with the exception of earthworms and springtails that were less than 1 mm.

**Mesocosm Construction**

The mesocosms were constructed within 18.9L plastic buckets (Encore, Sandusky, OH), with a top diameter of 30 cm and a height of 44 cm. Each bucket had seven, 4 mm diameter, holes drilled into the bottom. The holes were arranged exactly the same on each bucket, as a template was created and used to drill the holes. After the holes were drilled, 5200 mL of sand (purchased from Gelinas, Orange, MA) was put into each bucket, creating a layer approximately 10 cm high. The sand facilitated drainage and acted as a barrier between the ground and the soil layers in the mesocosms. To replicate the natural soil horizons, a 5200 mL mineral soil layer was added, followed by a 5200 mL organic layer. The soil was collected from the Tom Swamp tract of Harvard Forest, approximately 2 km away from the study site. Tom Swamp tract soil is Brookfield loam, while Prospect Hill Tract, the site of the warming chambers, is Canton loam. The soils are both fine sandy loam, mesic Typic Dystrudepts (Soil Survey Staff, 2013). The soil was collected in 30 cm diameter cores, using a knife to cut a circular outline, and a shovel to remove the soil. The cores were then separated into two sections: the first was a combination of the O and A horizons, since the O horizon was shallow and difficult to separate from A, and the second consisted of B horizon (hereafter "organic soil" and "mineral soil," respectively). The soils were sieved separately through 4 mm mesh to remove large rocks and sticks. Due to their large volumes, the organic soil, mineral soil, and sand were separately homogenized using the cone and quarter method (Gerlach, Dobb, Raab, & Nocerino, 2002). This method involves piling the soil, separating it into four sections, and creating one new pile by recombining the four sections. In this study the cone and quarter method was done 5 times for each soil type. Between

sieving, manual mixing, and the cone and quarter method, the soil was well mixed. After homogenization, invertebrates were removed by hand. Microscopic invertebrates were not removed, but densities found in randomly selected subsamples were not significantly different from one another.

One red maple (*Acer rubrum*) sapling (15-25 cm in height) (Musser Forests, Inc., Indiana, PA) was planted in each bucket and allowed to acclimate in a greenhouse for five days. In 10 random mesocosms, a Thermochrom iButton temperature sensor and logger (Model DS1921G-F5#, Maxim/Dallas, Dallas, TX) was placed between each layer of sediment. A 10 cm diameter, 4.5 cm high PVC collar, necessary for measuring $CO_2$ using LI-6400 (described below), was placed in each mesocosm. The collar was inserted approximately 1 cm into the soil of each mesocosm.

Invertebrates were added in densities similar to the area surrounding the chambers (see "Invertebrates"). Each mesocosm received three millipedes, three earthworms, and 45 mL of fine leaf litter containing springtails and mites. Due to the small size of springtails and mites, they were not added by count. After the leaf litter was sieved through 1 mm mesh, primarily springtails, mites, and small pieces of leaf litter remained. This was all combined, mixed thoroughly, and then added by volume to the buckets. By sorting several subsamples of this litter, it was determined that there were approximately 20 springtails and 14 mites per 45 mL. Two wolf spiders were added to half of the mesocosms (predator treatment). The escape or addition of new invertebrates to the mesocosms over the course of the experiment was prevented by a 1 mm mesh fabric covering the whole mesocosm, and tied with a rubber band to allow access during measurements. Finally, to mimic natural forest floor settings, an additional 2 g of dried leaf litter lacking fauna was added to each mesocosm. This litter was collected from litter

baskets in the area surrounding the warming chambers. See Figure 3 for a diagram of the mesocosm setup.

**Experimental Design**

Treatments involved the manipulation of predator presence (2 levels) and temperature (10 levels), resulting in 20 treatments (Table 1). Each treatment was replicated three times, for a total of 60 mesocosms. To produce the temperature treatments, mesocosms were housed in 10 different open-top warming chambers at Harvard Forest, Petersham, MA, thus true replication of each treatment was n=1, but designed for regression analysis across the 10 warming levels. Each week, $CO_2$ efflux was measured using a LI-6400 (LICOR, Lincoln, NE) with the soil respiration attachment. During the $CO_2$ measurements, plant height, leaf number, spider presence, air temperature, soil temperature, and soil moisture were recorded. During each measurement, the LI-6400 recorded three efflux measurements, which were averaged to produce an efflux reading for the measurement day. Three times a week, soil water was collected from lysimeters. Mesocosms were harvested during two different time periods (one replicate of each treatment on the first date, the remaining two of each the second date). This allowed for measurement of invertebrate depth, invertebrate community, decomposition, and microbial biomass at different time points.

At all times during the experiment, moisture was kept above 10% volumetric water content (VWC) to match average water content of the soil in the surrounding area, which was determined by averaging several subsamples taken surrounding the warming chambers, and to prevent water from becoming a limiting factor (Peralta, Ludmer, & Kent, 2013). The presence of spider predators was also kept constant; a replacement was added if a spider could not be located or was found dead during weekly measurements. After two and a half weeks, the spiders were

reduced to one per mesocosm due to concerns that spiders were preying on one another. Reduction to one spider did not appear to influence $CO_2$: linear models assessing the effect of predators on $CO_2$ efflux were not significantly different between the two time periods. The maple saplings were nearly all dead (72%) after 4 weeks and were cut at the soil level in all mesocosms following the first harvest to ensure that all mesocosms were under similar conditions. The maple saplings were received in a stressed condition and many may have failed to recuperate.

**Harvest**

The mesocosms were harvested at two different time points, one on 7/10/2014, 5 weeks after the experiment began and another on 9/10/2014, 14 weeks after the experiment began. During the first harvest, one replicate of each treatment was randomly selected. During the second harvest, the remaining two replicates of each treatment were harvested. The harvest of the buckets took place at the research site, directly outside the chambers. First, spiders were collected in vials. After spider collection, two 2 cm soil cores were taken for microbial biomass analysis. Leaf litter was removed, followed by the first centimeter of soil (surface soil), and remaining soil was removed 4 cm at a time, each layer stored separately.

Leaf litter was dried in a Berlese funnel and invertebrates were collected in buckets below the funnel. Each soil depth was searched completely for worms and millipedes, three 15 mL subsamples were taken from each depth and stored in ethanol for further invertebrate extraction under magnification. Three 15 mL subsamples were also taken from the surface soil and stored in ethanol. For each subsample, the invertebrates were counted and identified as

belonging to the taxa described in "Invertebrates." All invertebrates not belonging to those taxa, which may have been erroneously left in the soil or entered the mesocosm during the experiment, were identified to Order level. Since the mesocosms were moved only a few feet during the harvest, invertebrates would have had little chance to move, and therefore depth measurements should accurately represent actual invertebrate depth instead of a response to disturbance.

Microbial biomass (C and N) was determined by fumigation-extraction, using the protocol described in Weintraub, Scott-Denton, Schmidt, and Monson (2007). Five grams of homogenized soil from each mesocosm had 25 mL of .5 M potassium sulfate ($K_2SO_4$) added, and was mixed on a shaker table at 120 rpm for one hour. After shaking, the samples were vacuum filtered through Pall A/E glass fiber filters and frozen at -18°C. Alongside the $K_2SO_4$ extraction, 5 g of homogenized soil was fumigated by adding two milliliters of chloroform and incubated for 24 hours, followed by 30 minutes of venting. They were extracted using the same process described previously. The extracts were analyzed for DOC on a Shimadzu TOC-$V_{CPN}$ analyzer (Shimadzu Scientific Instruments Inc. Columbia, MD, USA). Microbial biomass carbon was determined by subtracting dissolved organic carbon (DOC) in the unfumigated samples and in the controls from the DOC in fumigated samples. This process was repeated for dissolved organic nitrogen (DON) to determine microbial nitrogen biomass.

**Data Analysis**

All analyses were done in R (R Core Team, 2013) using the nlme package (Pinheiro, Bates, DebRoy , Sarkar, & R Core Team, 2015). A longitudinal linear mixed effects model (LME) was used to determine the effect of predator presence, warming, their interaction, and other potential explanatory variables (e.g. microbial biomass, springtail density) on $CO_2$ efflux.

Warming was determined by calculating the mean temperature of the chamber (measured hourly), over the course of the experiment and subtracting the mean temperature of the control chamber. Mixed effects models were used in this study since they provide a way to include nested effects as well as autocorrelation in the model itself. Mixed effects models also can be used to analyze unbalanced data, as is produced in this data set by harvesting mesocosms through time. Mesocosm and chamber effects were accounted for by nesting mesocosm within chamber as a random effect. This random effects structure was directly based on the experimental design. An auto-regressive correlation structure of order 1 described the autocorrelation of weekly efflux measurements taken from the same mesocosm. An independent error structure was used to allow variance to differ for different dates when it significantly improved fit of the model, which was assessed using log-likelihood ratio tests. After developing the random effects structure, backwards model selection with log-likelihood ratio tests was used to determine the best fixed effects structure. The same process was employed to explore the effect of warming and predator presence on microbial biomass using LME. In addition to looking at microbial biomass as a potential predictor of efflux in the previous models, microbial biomass was considered as a predictor for only the last efflux measurement. Since microbial biomass was only measured during harvest, this analysis addressed the possibility that microbial biomass was not stable throughout the experiment, and therefore could predict only the last efflux reading. Separate one way ANOVAs were used to look at the effects of warming and predator presence on invertebrate density and earthworm burrowing depth.

The effects of warming, predator presence, and their interaction on warming on respiration was modeled for three different time periods, using three separate models. One model was created for the first four weeks, before the first harvest, using the data for all mesocosms

(hereafter "pre-harvest model"), another modeled data for only the two replicates remaining after the first harvest, from five weeks until the end of the experiment (hereafter "post-harvest model"). A third model incorporated data for all mesocosms throughout the entire experiment (hereafter "full experiment model"). The data were partitioned in this way for two reasons: mesocosm experiments require stabilization periods, with effects only evident after some time has passed (Pelini et al., In Review; Sitvarin & Rypstra, 2014) and shortly after the first harvest, plants were removed (see "Experimental Design"). Time could have been included as a fixed effect in a full experiment model, but since after the first harvest one replicate was removed, the change in sample size may have masked effects by giving pre-harvest measurements where the plant was present and mesocosms were not yet stabilized more weight. A marginal and conditional $R^2$ was calculated for each LME model using methods described by Nakagawa and Schielzeth (2013). Marginal $R^2$ is the proportion of the variance explained by only the fixed effects, while conditional $R^2$ is the proportion of the variance explained by both the fixed and random effects.

## RESULTS

For the pre-harvest period and for data taken from the full experiment, $CO_2$ efflux was not significantly predicted by warming, predator presence, their interaction, or any other variables measured in this study. However, during the post-harvest period, the best fit model predicted $CO_2$ efflux by warming, predator presence, and their interaction ($L$=5.90, df=8, p<.01; marginal $R^2$= .02, conditional $R^2$=.03; Figure 4). When no predator was present, $CO_2$ efflux increased with warming. However, when predators were present, $CO_2$ efflux did not increase with warming. In addition to the autocorrelation structure and nested random effects required by the experimental design (see "Methods: Data Analysis"), the post-harvest model also included an error structure that allowed variance to differ for each date ($L$=7.38, df=12, p<.01). Microbial biomass was not predicted by warming, predator presence, their interaction, or any other variables collected in this study (Figure 5). The last efflux measurement was not predicted by microbial biomass. Invertebrate density and earthworm burrowing depth did not differ between treatments (Figure 6).

## DISCUSSION

$CO_2$ efflux was significantly predicted by warming, predator presence, and their interaction during the last five weeks of the experiment. When predators were present, $CO_2$ efflux did not increase under warming as it did in the absence of predators. This finding suggests that predator presence may impact the effect of warming on detrital food webs and soil ecosystem functioning. This is contrary to our hypothesis; though there was an interactive effect of predator presence and warming, the presence of predators resulted in decreased respiration under warming, rather than the predicted increase.

The data suggest that the interaction of warming and predator presence on $CO_2$ efflux was not the result of consumptive effects. Soil microbial biomass C was not affected by warming or predator presence (Figure 5). However, a change in microbial $CO_2$ production may be due to altered rate of microbial processes that release $CO_2$ rather than a change in microbial biomass, since the microbial processes that release $CO_2$ are temperature sensitive (Davidson & Janssens, 2006). In addition, temperature sensitivity of microbial $CO_2$ release is dependent on many other factors not measured in this experiment, such as quality of the substrate and the composition of the microbial community (Davidson & Janssens, 2006; Fierer, Craine, McLauchlan, & Schimel, 2005). There was also no evidence of changes in invertebrate densities (e.g. earthworm, springtails, potworms, mites) (Figure 6), which indicates that predator presence did not reduce respiration via a direct consumptive pathway.

Predator presence likely reduced respiration response to warming via a non-consumptive pathway; although not through earthworms, since measurements of earthworm behavior (i.e. burrowing depth) were not significantly different between treatments. Though this study does not

have data to draw conclusions about the other invertebrates' behavioral response to predator presence, particularly mesofauna, there is evidence of non-consumptive predator effects in other studies, not only in the invertebrate herbivore community (Preisser & Strong, 2004; Strickland et al., 2013), but also in detritivores (Sitvarin & Rypstra, 2014; Zhao et al., 2013). In fact, a recent study by Sitvarin and Rypstra (2014) found that a wolf spider in the same genus as the one used in this experiment, *Pardosa spp.*, decreased $CO_2$ efflux in mesocosms colonized by springtails and held at 25°C, both when the spider was present and when only its cues were present. They suggest that activity of springtails is reduced due to fear effects, which have been shown in springtails exposed to predatory mites (Nilsson & Bengtsson, 2004) and in response to cues from recently deceased springtails of the same species (Sitvarin, Romanchek, & Rypstra, 2015). Since springtail presence has been shown to increase soil respiration (Fox et al., 2006), their altered activity could result in a reduction of $CO_2$ efflux. In addition, if detritivores are shifting their food preference, as has been observed in herbivores (Schmitz, 2006), microbe community structure may have changed (Crowther et al., 2011; Ronn et al., 2002; D. A. Wardle, 2006). However, the shift in Schmitz (2006) was largely due to the prey changing their microhabitat (e.g. type of plant) for shelter from predation, which may not have an analogue in the litter habitat. The interaction of predator presence and warming observed in this study could result if the predators are becoming more active at higher temperatures (Chown & Nicolson, 2004) and amplifying non-consumptive effects, whether the effect results from one of the examples given above or another mechanism entirely.

The contributions of predator presence to $CO_2$ efflux response to warming varied through time. The relationship is only apparent after week five, during the post-harvest time period. This may be due to mesocosm stabilization or plant presence masking predator and warming effects

during the pre-harvest time period. Timing of when most plants died (see "Methods: Experimental Design") falls within a reasonable range of time when the mesocosms may have been stabilizing (Pelini et al., In Review). The shared timing of these events makes drawing conclusions about why the pattern did not emerge until week five difficult. It is possible that the death of the plant may have released the system from bottom-up control, changed the microclimate in the mesocosm, or reduced substrate for microbes, since plant roots and their exudates provide material to microbes for break down (Lavelle, 1997; Newman & Watson, 1977). Even considering these challenges for interpretation of the results, the interaction of predation and warming is highly significant and likely not spurious.

This study provides insight into the role of soil invertebrates in the carbon cycle, the extent of which is not yet known and is in need of further research (O. J. Schmitz et al., 2014). The results suggest that generalist invertebrate predators may reduce $CO_2$ efflux in detrital food webs under warming. These findings could have implications for climate change mitigation strategies. Conservation efforts that work to maintain complete detrital food webs in deciduous forests could reduce soil $CO_2$ efflux, reducing feedback effects of climate change and warming. Protecting predators is particularly important since higher trophic levels are more sensitive to warming (Menge & Sutherland, 1987; Preisser & Strong, 2004; Voigt et al., 2003). The results of this study warrant future research into mechanistic explanations and generality of the effect to other systems.

## REFERENCES

A'Bear, A. D., Boddy, L., & Jones, T. H. (2013). Bottom-up determination of soil collembola diversity and population dynamics in response to interactive climatic factors. *Oecologia, 173*(3), 1083-1087. doi: 10.1007/s00442-013-2662-3

Barton, B. T., Beckerman, A. P., & Schmitz, O. J. (2009). Climate warming strengthens indirect interactions in an old-field food web. *Ecology, 90*(9), 2346-2351. doi: 10.1890/08-2254.1

Blankinship, J. C., Niklaus, P. A., & Hungate, B. A. (2011). A meta-analysis of responses of soil biota to global change. *Oecologia, 165*(3), 553-565. doi: 10.1007/s00442-011-1909-0

Bokhorst, S., Huiskes, A., Convey, P., van Bodegom, P. M., & Aerts, R. (2008). Climate change effects on soil arthropod communities from the Falkland Islands and the Maritime Antarctic. *Soil Biology & Biochemistry, 40*(7), 1547-1556. doi: 10.1016/j.soilbio.2008.01.017

Briones, M. J. I., Ostle, N. J., McNamara, N. R., & Poskitt, J. (2009). Functional shifts of grassland soil communities in response to soil warming. *Soil Biology & Biochemistry, 41*(2), 315-322. doi: 10.1016/j.soilbio.2008.11.003

Chown, S., & Nicolson, S. (2004). *Insect physiological ecology: mechanisms and patterns*. Oxford: Oxford University Press.

Crowther, T. W., Jones, T. H., & Boddy, L. (2011). Species-specific effects of grazing invertebrates on mycelial emergence and growth from woody resources into soil. *Fungal Ecology, 4*(5), 333-341. doi: 10.1016/j.funeco.2011.05.001

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature, 440*(7081), 165-173. doi: 10.1038/nature04514

De Vries, F. T., Thebault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. A., Bjornlund, L., . . . Bardgett, R. D. (2013). Soil food web properties explain ecosystem services across European land use systems. *Proceedings of the National Academy of Sciences of the United States of America, 110*(35), 14296-14301. doi: 10.1073/pnas.1305198110

Del Toro, I., Ribbons, R. R., & Ellison, A. M. (2015). Ant–mediated ecosystem functions on a warmer planet: effects on soil movement, decomposition and nutrient cycling. *Journal of Animal Ecology*, n/a-n/a. doi: 10.1111/1365-2656.12367

Fierer, N., Craine, J. M., McLauchlan, K., & Schimel, J. P. (2005). Litter quality and the temperature sensitivity of decomposition. *Ecology, 86*(2), 320-326. doi: 10.1890/04-1254

Fox, O., Vetter, S., Ekschmitt, K., & Wolters, V. (2006). Soil fauna modifies the recalcitrance-persistence relationship of soil carbon pools. *Soil Biology & Biochemistry, 38*(6), 1353-1363. doi: 10.1016/j.soilbio.2005.10.014

Gerlach, R. W., Dobb, D. E., Raab, G. A., & Nocerino, J. M. (2002). Gy sampling theory in environmental studies. 1. Assessing soil splitting protocols. *Journal of Chemometrics, 16*(7), 321-328. doi: 10.1002/cem.705

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., & Hattenschwiler, S. (2010). Diversity meets decomposition. *Trends in Ecology & Evolution, 25*(6), 372-380.

Hanson, P. J., Edwards, N. T., Garten, C. T., & Andrews, J. A. (2000). Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry, 48*(1), 115-146. doi: 10.1023/a:1006244819642

Hawlena, D., Strickland, M. S., Bradford, M. A., & Schmitz, O. J. (2012). Fear of Predation Slows Plant-Litter Decomposition. *Science, 336*(6087), 1434-1438. doi: 10.1126/science.1220097

Heemsbergen, D. A., Berg, M. P., Loreau, M., van Haj, J. R., Faber, J. H., & Verhoef, H. A. (2004). Biodiversity effects on soil processes explained by interspecific functional dissimilarity. *Science, 306*(5698), 1019-1020. doi: 10.1126/science.1101865

IPCC. (2013). Climate change 2013: the physical science basis. Working group I contribution to the fifth assessment report of the intergovernmental panel on climate change. In T. F. Stocker, D. Qin, G. K. Plattner, M. M. B. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex & P. M. Midgley (Eds.), (pp. 1-14). Cambridge, UK.

Jochum, M., Schneider, F. D., Crowe, T. P., Brose, U., & O'Gorman, E. J. (2012). Climate-induced changes in bottom-up and top-down processes independently alter a marine ecosystem. *Philosophical Transactions of the Royal Society B-Biological Sciences, 367*(1605), 2962-2970. doi: 10.1098/rstb.2012.0237

Lavelle, P. (1997). Faunal activities and soil processes: Adaptive strategies that determine ecosystem function. *Advances in Ecological Research, Vol 27, 27*, 93-132. doi: 10.1016/s0065-2504(08)60007-0

Menge, B. A., & Sutherland, J. P. (1987). Community regulation-variation in disturbance, competition, and predation in relation to environmental-stress recruitment. . *American Naturalist, 130*(5), 730-757. doi: 10.1086/284741

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution, 4*(2), 133-142. doi: 10.1111/j.2041-210x.2012.00261.x

Newman, E. I., & Watson, A. (1977). Microbial abundance in rhizosphere-computer-model. *Plant and Soil, 48*(1), 17-56. doi: 10.1007/bf00015157

Nielson, U. N., Ayres, E., Wall, D. H., & Bardgett, R. D. (2011). Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity-function relationships. *European Journal of Soil Science, 62*, 105-116. doi: 10.1111/j.1365-2389.2010.01314.x

Nilsson, E., & Bengtsson, G. (2004). Death odour changes movement pattern of a Collembola. *Oikos, 104*(3), 509-517. doi: 10.1111/j.0030-1299.2004.12921.x

Pelini, S. L., Diamond, S. E., Nichols, L. M., Stuble, K. L., Ellison, A. M., Sanders, N. J., . . . Gotelli, N. J. (2014). Geographic differences in effects of experimental warming on ant species diversity and community composition. *Ecosphere, 5*(10), art125. doi: 10.1890/ES14-00143.1

Pelini, S. L., Maran, A. M., Chen, A., Kaseman, J., & Crowther, T. W. (In Review). Untangling the indirect effects of climate change on terrestrial ecosystem functioning.

Peralta, A. L., Ludmer, S., & Kent, A. D. (2013). Hydrologic history influences microbial community composition and nitrogen cycling under experimental drying/wetting treatments. *Soil Biology & Biochemistry, 66*, 29-37. doi: 10.1016/j.soilbio.2013.06.019

Pinheiro, J., Bates, D., DebRoy , S., Sarkar, D., & R Core Team. (2015). Linear and Nonlinear Mixed Effects Models (Version 3.1-120). Retrieved from http://CRAN.R-project.org/package=nlme

Preisser, E. L., Bolnick, D. I., & Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology, 86*(2), 501-509. doi: 10.1890/04-0719

Preisser, E. L., & Strong, D. R. (2004). Climate affects predator control of an herbivore outbreak. *American Naturalist, 163*(5), 754-762. doi: 10.1086/383620

R Core Team. (2013). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org.

Rantalainen, M. L., Fritze, H., Haimi, J., Kiikkila, O., Pennanen, T., & Setala, H. (2004). Do enchytraeid worms and habitat corridors facilitate the colonisation of habitat patches by soil microbes? *Biology and Fertility of Soils, 39*(3), 200-208. doi: 10.1007/s00374-003-0687-1

Ronn, R., McCaig, A. E., Griffiths, B. S., & Prosser, J. I. (2002). Impact of protozoan grazing on bacterial community structure in soil microcosms. *Applied and Environmental Microbiology, 68*(12), 6094-6105. doi: 10.1128/aem.68.12.6094-6105.2002

Schlesinger, W. H., & Andrews, J. A. (2000). Soil respiration and the global carbon cycle. *Biogeochemistry, 48*(1), 7-20. doi: 10.1023/a:1006247623877

Schmitz. (2006). Predators have large effects on ecosystem properties by changing plant diversity, not plant biomass. *Ecology, 87*(6), 1432-1437. doi: 10.1890/0012-9658(2006)87[1432:phleoe]2.0.co;2

Schmitz, O. J., Raymond, P. A., Estes, J. A., Kurz, W. A., Holtgrieve, G. W., Ritchie, M. E., . . . Wilmers, C. C. (2014). Animating the Carbon Cycle. *Ecosystems, 17*(2), 344-359. doi: 10.1007/s10021-013-9715-7

Sitvarin, M. I., Romanchek, C., & Rypstra, A. L. (2015). Nonconsumptive Predator–Prey Interactions: Sensitivity of the Detritivore Sinella curviseta (Collembola: Entomobryidae) to Cues of Predation Risk From the Spider Pardosa milvina (Araneae: Lycosidae). *Environmental Entomology*.

Sitvarin, M. I., & Rypstra, A. L. (2014). Fear of predation alters soil carbon dioxide flux and nitrogen content. *Biology Letters, 10*(6), 4. doi: 10.1098/rsbl.2014.0366

Soil Survey Staff. (2013). Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Retrieved 12/29/2014

Strickland, M. S., Hawlena, D., Reese, A., Bradford, M. A., & Schmitz, O. J. (2013). Trophic cascade alters ecosystem carbon exchange. *Proceedings of the National Academy of Sciences of the United States of America, 110*(27), 11035-11038. doi: 10.1073/pnas.1305191110

Strickland, M. S., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Testing the functional significance of microbial community composition. *Ecology, 90*(2), 441-451. doi: 10.1890/08-0296.1

Voigt, W., Perner, J., Davis, A. J., Eggers, T., Schumacher, J., Bahrmann, R., . . . Sander, F. W. (2003). Trophic levels are differentially sensitive to climate. *Ecology, 84*(9), 2444-2453. doi: 10.1890/02-0266

Wall, D., Bradford, M., St. John, M., & et.al. (2008). Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. *Global Change Biology, 14*, 2661–2677. doi: doi: 10.1111/j.1365-2486.2008.01672.x

Wardle, D. A. (2002). *Communities and ecosystems: Linking the aboveground and belowground components* (Vol. 34). Princeton, New Jersey: Princeton University Press.

Wardle, D. A. (2006). The influence of biotic interactions on soil biodiversity. *Ecology Letters, 9*(7), 870-886. doi: 10.1111/j.1461-0248.2006.00931.x

Weintraub, M. N., Scott-Denton, L. E., Schmidt, S. K., & Monson, R. K. (2007). The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient

availability in a subalpine forest ecosystem. *Oecologia, 154*(2), 327-338. doi: 10.1007/s00442-007-0804-1

Wieder, W. R., Bonan, G. B., & Allison, S. D. (2013). Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change, 3*(10), 909-912. doi: 10.1038/nclimate1951

Wimp, G. M., Murphy, S. M., Lewis, D., Douglas, M. R., Ambikapathi, R., Van-Tull, L., . . . Denno, R. F. (2013). Predator hunting mode influences patterns of prey use from grazing and epigeic food webs. *Oecologia, 171*(2), 505-515. doi: 10.1007/s00442-012-2435-4

Zhang, S. X., Li, Q., Lu, Y., Zhang, X. P., & Liang, W. J. (2013). Contributions of soil biota to C sequestration varied with aggregate fractions under different tillage systems. *Soil Biology & Biochemistry, 62*, 147-156. doi: 10.1016/j.soilbio.2013.03.023

Zhao, C., Griffin, J. N., Wu, X., & Sun, S. (2013). Predatory beetles facilitate plant growth by driving earthworms to lower soil layers. *Journal of Animal Ecology, 84*(4), 749-758. doi: 10.1111/1365-2656.12058

**Table 1.** Summary of treatments included in the experiment. Each treatment was replicated three times

| Predator presence/absence | Warming above ambient (°C) |
|---|---|
| P | 0° |
| A | 0° |
| P | +1.5° |
| A | +1.5° |
| P | +2° |
| A | +2° |
| P | +2.5° |
| A | +2.5° |
| P | +3° |
| A | +3° |
| P | +3.5° |
| A | +3.5° |
| P | +4° |
| A | +4° |
| P | +4.5° |
| A | +4.5° |
| P | +5° |
| A | +5° |
| P | +5.5° |
| A | +5.5° |

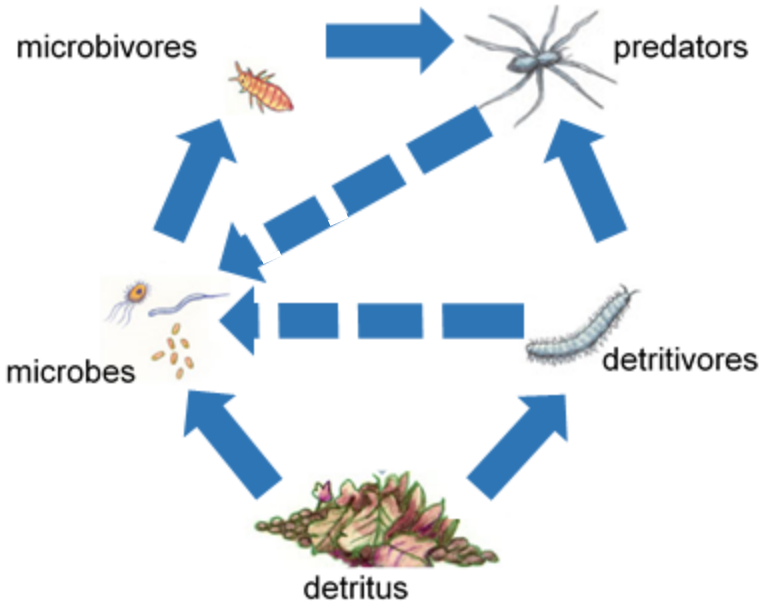


**Figure 1.** Belowground Food Web**.** Unbroken arrows signify direct effects, broken arrows signify indirect effects. Figure credit: Shannon Pelini

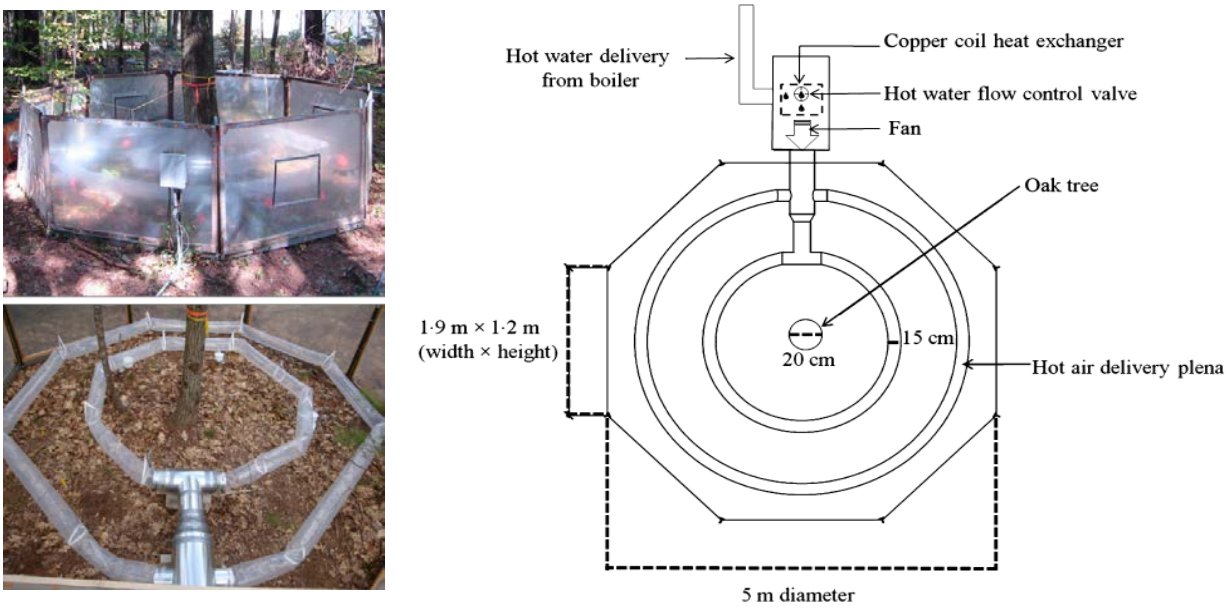


**Figure 2.** Warming chamber diagram, view from above. From Pelini et. al, 2011**.**

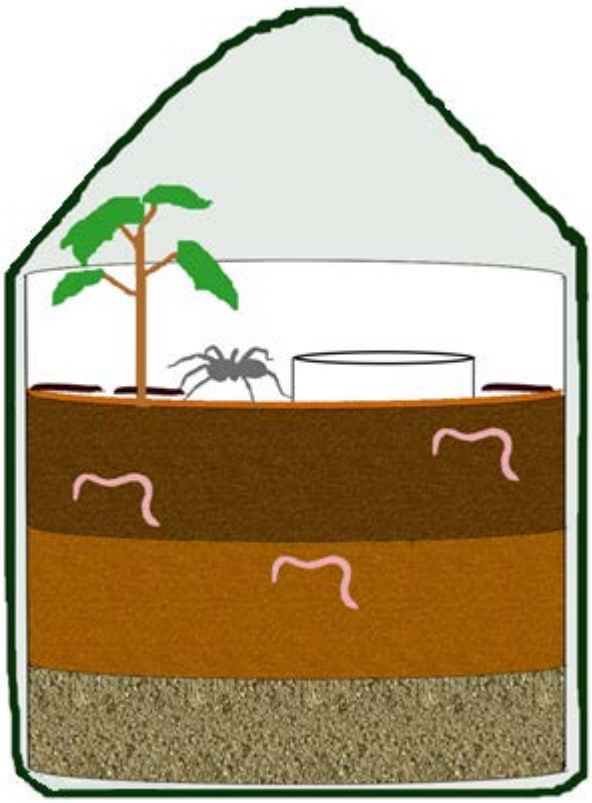

**Figure 3.** Diagram of mesocosm**.** From top to bottom, each mesocosm contained a layer of leaf litter, organic soil, mineral soil, and sand. One PVC soil collar was added to each mesocosm, as well as three earthworms, three millipedes, and one red maple sapling. Those mesocosms belonging to the predator present treatment had one wolf spider added. Each mesocosm was enclosed in fine mesh.

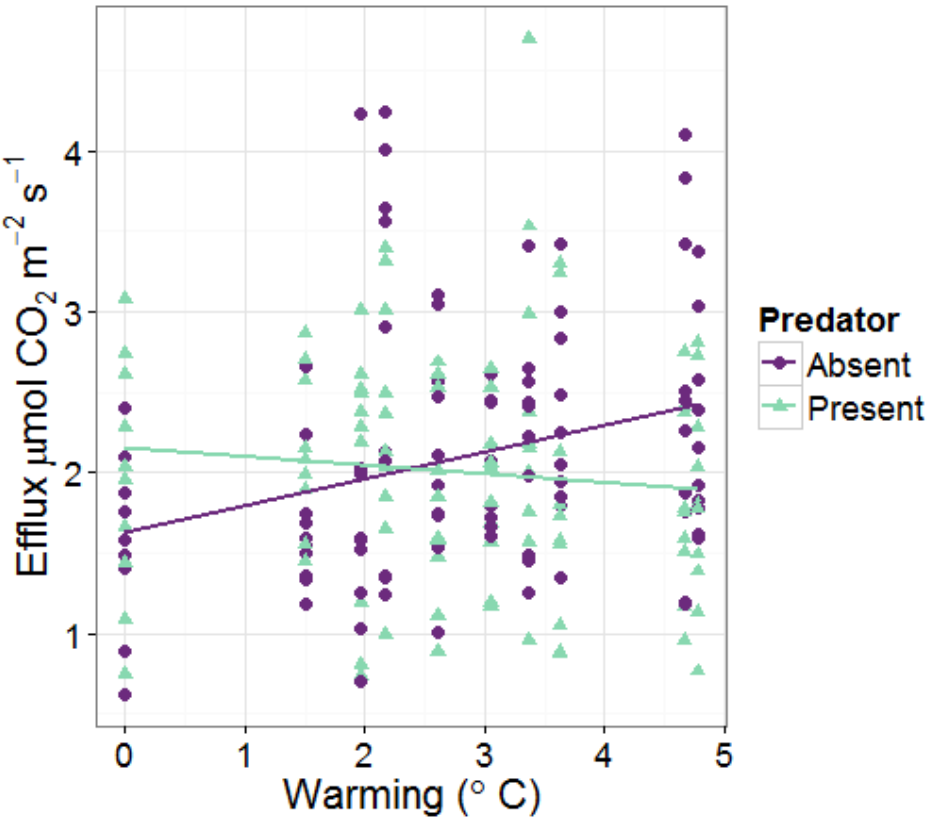


**Figure 4.** $CO_2$ efflux as predicted by warming, predator presence, and their interaction during post-harvest time period. Trend lines are predicted by the post-harvest model.

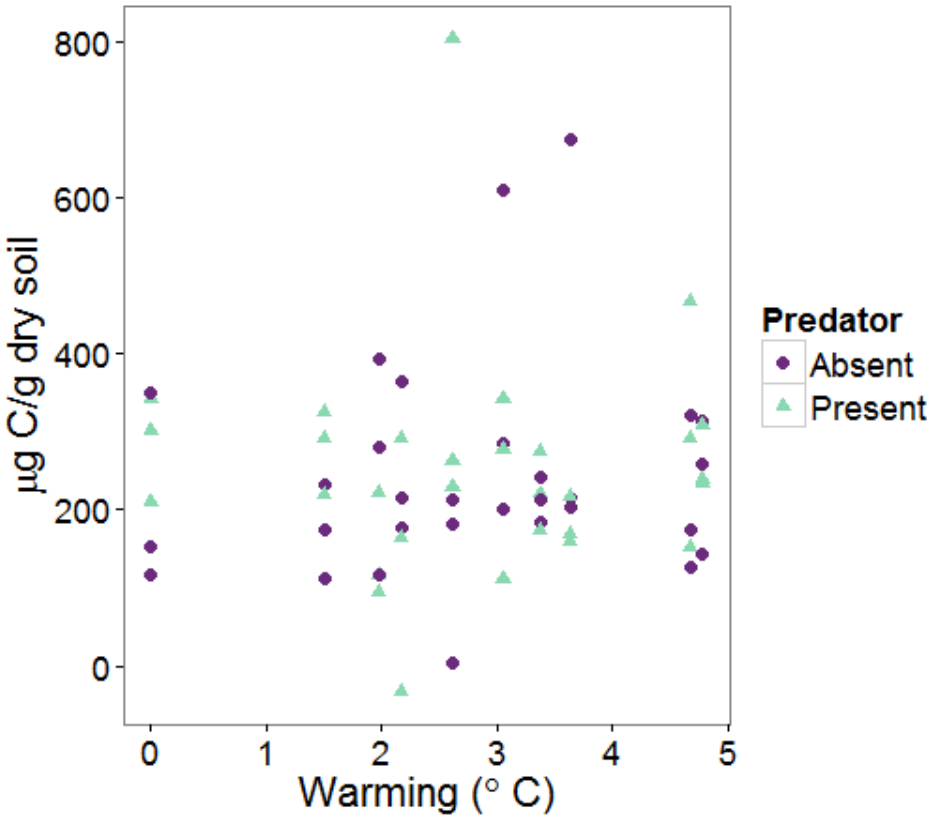


**Figure 5.** Microbial biomass carbon as predicted by warming, predator presence, and their interaction at the time of harvest.

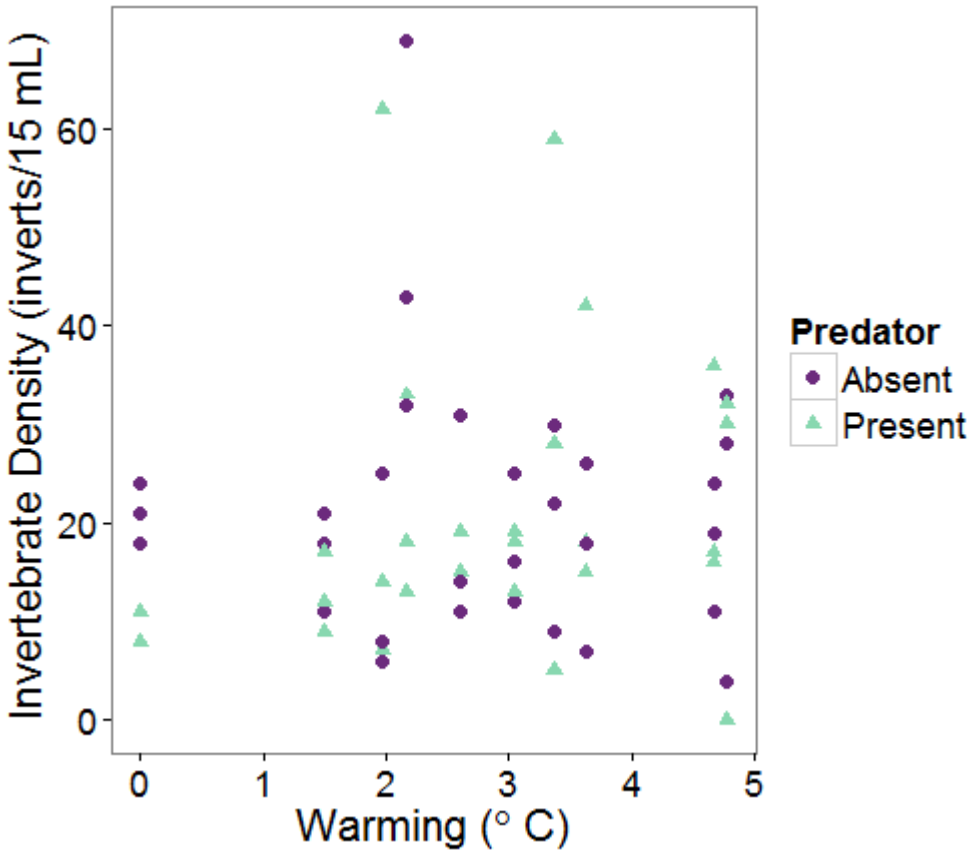


**Figure 6.** Invertebrate density as predicted by warming, predator presence, and their interaction at the time of harvest.