Video Article

Linking Predation Risk, Herbivore Physiological Stress and Microbial Decomposition of Plant Litter

Oswald J. Schmitz[1], Mark A. Bradford[1], Michael S. Strickland[1,2], Dror Hawlena[3]

[1]School of Forestry and Environmental Studies, Yale University

[2]Department of Biological Sciences, Virginia Tech

[3]Department of Ecology, Evolution and Behavior, The Hebrew University of Jerusalem

Correspondence to: Oswald J. Schmitz at oswald.schmitz@yale.edu

Abstract

The quantity and quality of detritus entering the soil determines the rate of decomposition by microbial communities as well as recycle rates of nitrogen (N) and carbon (C) sequestration[1,2]. Plant litter comprises the majority of detritus[3], and so it is assumed that decomposition is only marginally influenced by biomass inputs from animals such as herbivores and carnivores[4,5]. However, carnivores may influence microbial decomposition of plant litter via a chain of interactions in which predation risk alters the physiology of their herbivore prey that in turn alters soil microbial functioning when the herbivore carcasses are decomposed[6]. A physiological stress response by herbivores to the risk of predation can change the C:N elemental composition of herbivore biomass[7,8,9] because stress from predation risk increases herbivore basal energy demands that in nutrient-limited systems forces herbivores to shift their consumption from N-rich resources to support growth and reproduction to C-rich carbohydrate resources to support heightened metabolism[6]. Herbivores have limited ability to store excess nutrients, so stressed herbivores excrete N as they increase carbohydrate-C consumption[7]. Ultimately, prey stressed by predation risk increase their body C:N ratio[7,10], making them poorer quality resources for the soil microbial pool likely due to lower availability of labile N for microbial enzyme production[6]. Thus, decomposition of carcasses of stressed herbivores has a priming effect on the functioning of microbial communities that decreases subsequent ability to of microbes to decompose plant litter[6,10,11].

We present the methodology to evaluate linkages between predation risk and litter decomposition by soil microbes. We describe how to: induce stress in herbivores from predation risk; measure those stress responses, and measure the consequences on microbial decomposition. We use insights from a model grassland ecosystem comprising the hunting spider predator (*Pisuarina mira*), a dominant grasshopper herbivore (*Melanoplus femurrubrum*),and a variety of grass and forb plants[9].

Video Link

The video component of this article can be found at https://www.jove.com/video/50061/

Protocol

1. Rearing Grasshoppers Under Stress and Stress Free Conditions

1. Use 0.5 m^2 circular, closed mesocosms to prevent emigration or immigration of animal species (**Figure 1**). Construct mesocosms using 2.4 m lengths of 1.5 m high ¼" mesh aluminum fence as a scaffolding. Cover the fencing with 2.5 m lengths 1.75 m high aluminum window screening folded over the top and bottom of the fencing and stapled together along fold. Join the fencing ends to form a closed circle and then staple the overlapping window screening together to create a seal. Set the mesocosm into the soil in the field by digging a 10 cm deep by 4 cm wide trench around the base of the mesocosom, sink the mesocosm into the trench and then tamp the sod of the trench around the sunken part of the mesocosm. Staple a circular piece of window screening to the top of the mesocosm.
2. Array mesocosms in a replicated paired experimental design in the field. Plot locations should be selected to match the plant species identity and plant relative cover. Sink cages 10 cm into the ground at the plot site.
3. Using a sweep net, collect early (2nd) instar grasshopper nymphs and stock them into the mesocosms at natural field densities.
4. Using a sweep net, capture individuals of a dominant sit-and-wait hunting (not web weaving) spider predator species. Glue shut the spider chelicerae (mouthparts used to subdue prey) with fast-drying cement to decoupled risk effects from actual survival selection favoring individual grasshoppers with better abilities to evade spider predation. Stock the spiders at field densities to one mesocosm of each pair. This will be the stress treatment. Mesocosms without spiders will be the stress free treatment.

5. Allow grasshopper nymphs to develop to late (4th and 5th) instar stages. Collect all individuals from the cages and randomly assign individuals from each cage to one of three subsequent assay groups: (1) validation of physiological stress state; (2) validation of shift in body elemental stoichiometry; (3) microbial decomposition.

2. Validating Grasshopper Stress State

1. Measure grasshopper standard metabolic rate (SMR), as the rate of carbon dioxide emission ($\dot{V}_{CO_2}$) in an incurrent flow-through respirometry system with an air flow rate of 200 ml/min. Remove water vapor by passing flowing air through a drying agent.
2. Following food deprivation of 16 hr (water should be available), weigh individual grasshoppers (±0.1 mg), and place them in transparent 50 ml (9.2 cm L x 2.0 cm D) respirometer chambers and allow them to recover from handling for at least 10 min before measurements commence.
3. Under constant average ambient temperature (temperature ± 1% standard error variation) within the respirometer chamber, analyze respired air using an infra-red CO_2 analyzer (*e.g.* Qubit S151- 1 ppm resolution). Measure the mean minimal steady-state $\dot{V}_{CO_2}$ for 10 min.
4. The analyzer provides fractional CO_2 concentration (parts-per-million), yet SMR should be reported as a rate, so one must transformed the recordings as $\dot{V}_{CO_2} = FR_i(F'_{e\,CO_2} - F_{i\,CO_2})/\{1 - F'_{e\,CO_2}[1-(1/RQ)]\}$, where

$F_i co_2$ = incurrent fractional concentration of CO_2;

$F_e co_2$ = excurrent fractional concentration of CO_2;   FR = flow rate (mL min^{-1});

RQ = respiratory quotient, assumed equal to 0.85 in herbivorous animals.

3. Validating Shift in Body Elemental Stoichiometry

1. Evaluate Carbon:Nitrogen (C:N) content of a sample of grasshoppers collected from the field mesocosms.
2. Reduce variation in C:N due to recent food consumption by removing grasshopper gut contents under a dissecting microscope.
3. Freeze-dry the empty gut and body for 48 hr and then grind the individual carcass and gut to a homogeneous powder.
4. Measure C:N contents of the powder using a CNH autoanalyzer.

4. Microbial Decomposition

1. Place replicated pairs of PVC collars (15.4-cm dia., inserted ~4 cm into the soil) in the field site (**Figure 3C**). Remove all vegetation within them via clipping at the soil surface. These collars are used for decomposition measures. Additionally, establish a set of PVC collars across the field site to act as ^{13}C natural-abundance controls (see below), to which neither grasshoppers nor grass-litter are added.
2. To one collar in each pair add 2 intact, freeze-dried carcasses of grasshoppers (record the biomass added) reared with predator risk as described above using field-cages. To the other collar in each pair add 2 intact freeze-dried carcasses reared without predator risk.
3. Cover the PVC collars with a screen to prevent grasshopper removal by scavengers from the plots and let the grasshopper carcasses decompose for 40 days.
4. While carcasses are decomposing, label grass-litter with ^{13}C.
   1. Construct a clear plexiglass chamber (60 cm x 60 cm x 1.5 m) with an inlet and outlet valve (**Figure 3B**).
   2. Sink a square 60 cm x 60 cm wooden frame with a rubber seal coated with silicon grease 5 cm into the ground (**Figure 3B**).
   3. Slide the chamber on top of the wooden frame so that the chamber becomes sealed by the rubber (**Figure 3B**).
   4. Connect the chamber inlets to compressed gas cylinders containing 99 atom% ^{13}CO$_2$. Plants inside the chamber will be labelled with ^{13}C, where CO_2 concentrations are maintained at ambient levels (because elevating concentrations alters plant carbon partitioning). Ambient levels are maintained by only pulsing labeled CO_2 for short periods of time. Also, chamber temperatures are monitored and chambers are removed if temperatures reach 5 °C above ambient.
   5. One week after labeling, compare δ^{13}C of the grass-litter with natural abundance values collected from a random sample of identical grass species using a Thermo DeltaPlus isotope ratio mass spectrometer (Thermo, San Jose, CA, USA).
5. After 40 days, add 10 g of air-dried ^{13}C-labeled grass-litter to each collar that had previously been amended with grasshopper carcasses.
6. Monitor the mineralization rate of the grass-litter *in situ* across 75 days by capping each collar and tracking both total soil respiration and the respiration of ^{13}CO$_2$. This is accomplished using a flow-through chamber technique where gas samples from each collar are monitored, in real time, for 8 min each using cavity ring-down spectroscopy (CRDS; Picarro Inc., Santa Clara, CA, USA; Model: G1101-i). CRDS enables one to simultaneously track both total and δ^{13}C of soil respiration.
7. Estimate the contribution of ^{13}C-labeled grass-litter to total soil respiration using isotope mixing equations. The amount of grass-litter derived CO_2 is calculated as follows: $C_{grass\text{-}litter\,derived} = C_{total} \times (\delta^{13}C_{respiration} - \delta^{13}C_{nat.abn.})/(\delta^{13}C_{grass\text{-}litter} - \delta^{13}C_{nat.abn})$, where C_{total} is the total amount of C respired during a given measurement, δ$^{13}C_{respiration}$ is the δ^{13}C of respired-C for collars amended with labeled grass litter, δ$^{13}C_{nat.abn.}$ is the mean δ^{13}C of respired C in the three natural abundance collars (*i.e.* those that were not amended with litter), and δ$^{13}C_{grass\text{-}litter}$ is the δ^{13}C of the grass litter added to the collars.
8. Monitor both soil temperature and moisture across the experiment using hand-held probes to correct for differences in soil respiration due to differences in temperature and moisture.

9. Although intended for field use, the cavity ring-down spectroscopy instrument (Picarro Inc., Santa Clara, CA, USA; Model: G1101-i) readings are sensitive to movement. Therefore, one should erect a base measurement station central to all of the plots containing PVC collars, and connect the instrument to the collars with lengths of PVC tubing.

Representative Results

An example plot of grasshopper standard metabolic rates in stress and stress free conditions are presented in **Figure 2**. Due to body mass differences among individual grasshoppers, and the fact that metabolic rate varies with body mass, plots should present metabolic rates in relation to grasshopper body mass. Parallel trends for the different treatments indicate that metabolic rate rises as a constant multiple of standard metabolic rate (*i.e.* there is no body mass x metabolic rate interaction) for all stressed individuals.

Grasshopper body C and N elemental contents in risk and risk free conditions are presented in **Table 1**. It is noteworthy that there is a very small (4%) difference in body C:N ratios between treatments. Nevertheless, these small differences can translate into large differences in grass litter decomposition by the soil microbial pool (**Figure 3**).

Adding grass litter to PVC collars previously amended with stressed or stress-free grasshoppers leads to different degrees of litter decomposition, as reflected in the curves describing cumulate CO_2 release from the soil due to microbial respiration (**Figure 3**). Experiments should be monitored until cumulate curves begin to saturate.

|	Stress	Stress Free
Carbon (%)	48.44±0.32	44.73±0.46
Nitrogen (%)	12.11±0.08	11.62±0.12
Carbon: Nitrogen	4.00±0.03	3.85±0.04

Table 1. Comparison of the chemical content of grasshopper herbivore carcasses from conditions in which they faced predation risk (stress) and in which predation risk was absent (stress free). Values are mean ± 1 standard error.
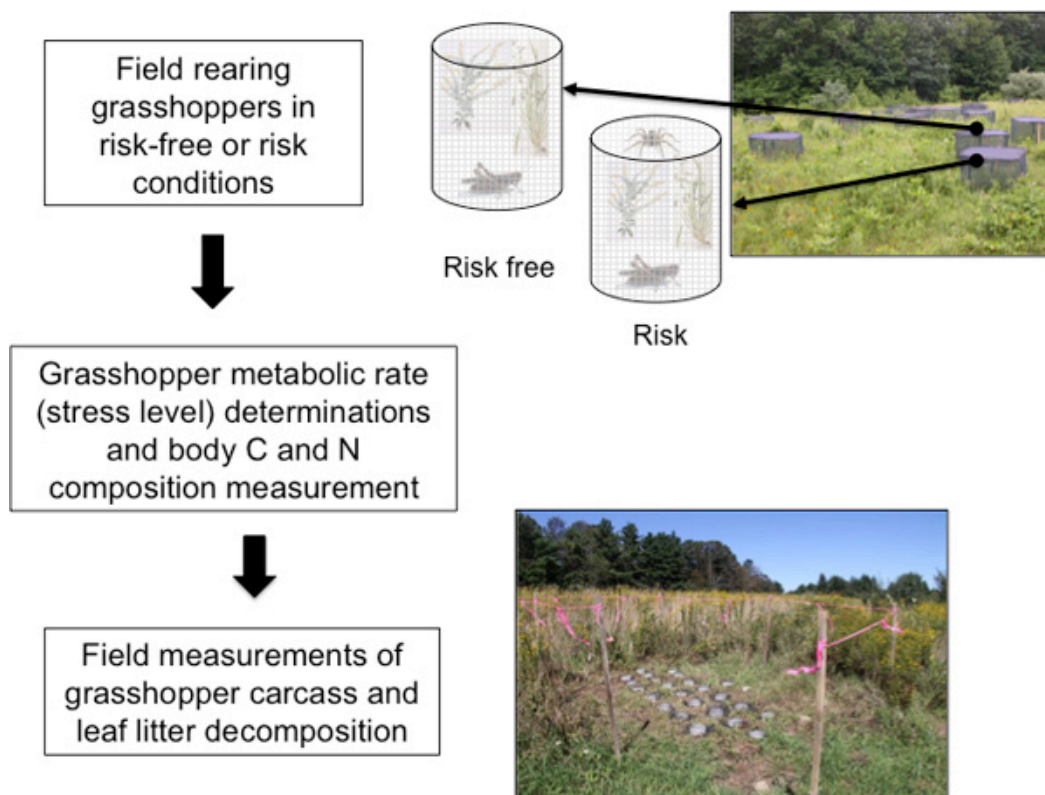
Figure 1. Illustration of the design of the field mesocosms used in the experiment and overall scheme of the experimental evaluation of risk effects on litter decomposition.
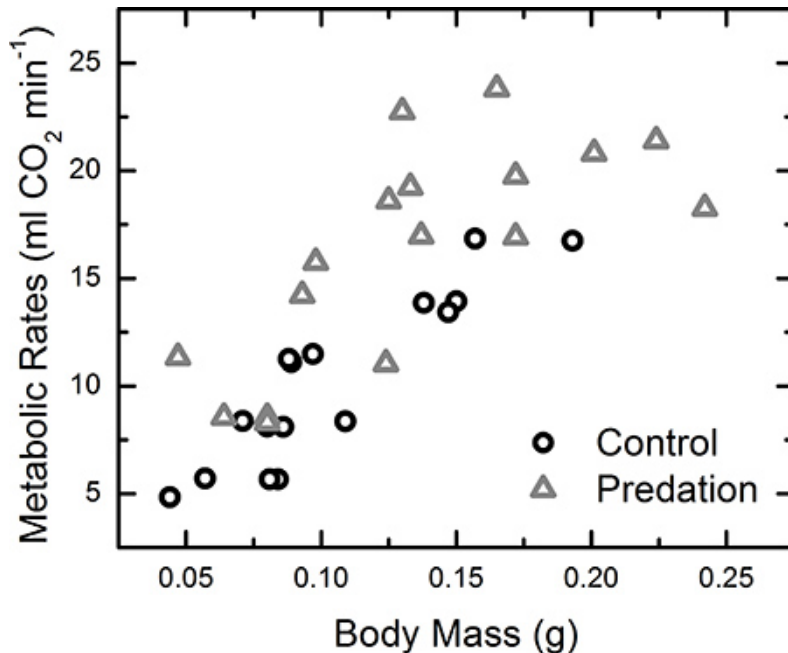
Figure 2. A plot of herbivore standard metabolic rate in relation to herbivore body mass. The data are divided into two classes according to experimental treatment: grasshoppers from mesocosms containing predators (predation) to induce stress, and mesocosms without predators (control) and hence no induced stress. Data are from D. Halwena and O.J. Schmitz 2010, unpublished.
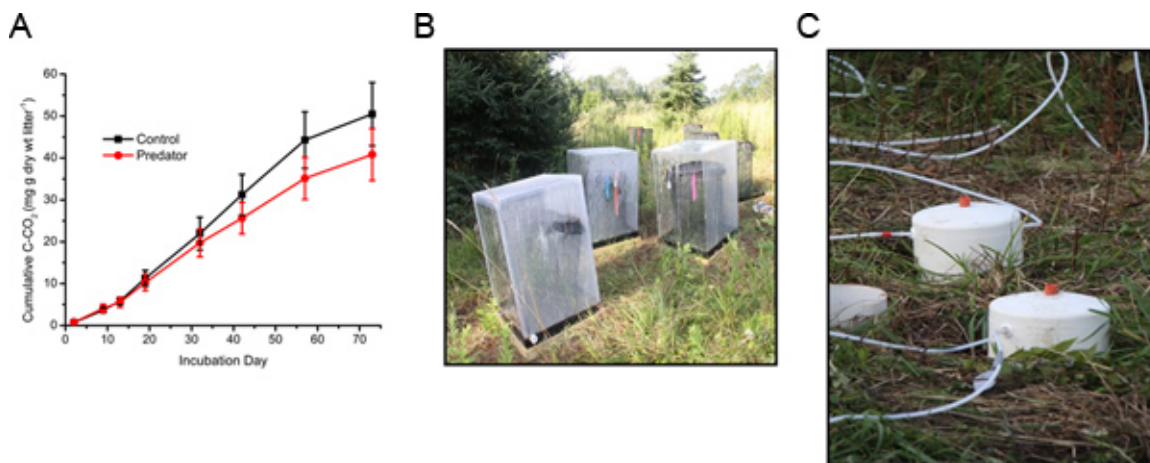
Figure 3. Curves describing cumulative CO_2 release by the microbial pool while decomposing experimental grass litter inputs in PVC collars. Plotted values are mean ± 1 standard error. The graph demonstrates that soils primed with stressed grasshopper carcasses (predator) result in 19% lower (ANOVA $F_{1,6} = 9.06$, $P < 0.05$) plant litter decomposition rates than soils primed with stress free grasshopper carcasses (control). The inset shows the PVC collar apparatus in the field. Figure reproduced from Hawlena *et al.*[6] Cick here to view larger figure.

Discussion

The sequence of methods presented here should allow systematic measurement of the way stress in species comprising above-ground food webs can prime soil microbial communities in ways that lead to alteration of subsequent decomposition of plant litter. The methods are ideal for studying ecosystems comprised of arthropod consumers and herbaceous plants because intact food webs can be spatially circumscribed and contained within mesocosms.

Spatial variability may exist due to gradients in background soil moisture, soil temperature, plant nutrient content, *etc.* The study design allows one to array mescosms and PVC collars to block along spatial environmental gradients and thereby account for such environmental variation when analyzing for effects.

Although intended for field use, the cavity ring-down spectroscopy instrument (Picarro Inc., Santa Clara, CA, USA; Model: G1101-i) readings are sensitive to movement. Therefore, one should erect a base measurement station central to all of the plots containing PVC collars, and connect the instrument to the collars with lengths of PVC tubing.

Soil litter decomposition has traditionally been measured by enclosing known quantities of litter into fiberglass mesh bags, depositing the bags onto the soil surface in the field and periodically re-measuring the bags to quantify litter disappearance rate (decomposition). The limitation

of this method is that one is unable to trace the fate of the decomposed matter or determine the contribution to CO_2 mineralization of the soil amendment (added litter) from background soil CO_2 mineralization. The tracer method using labeled CO_2 presented here helps alleviate this logistical constraint.

Ecosystem ecology and biogeochemistry have operated under the working paradigm that because uneaten plant-litter comprises the majority of detritus, belowground ecosystem processes are only marginally influenced by biomass inputs from higher trophic levels in aboveground food webs, such as herbivores themselves[6]. However, there is growing evidence that species in higher trophic levels of ecosystems can have a profound influence on belowground processes[1,4,5]. The method presented here stands to enhance quantification of the contribution of higher trophic levels, either directly through biomass from carcass deposition (*e.g.* [12, 13]) or excretion and egestion (*e.g.* [14, 15]) or indirectly through alteration of plant community composition (*e.g.* [9]) on ecosystem nutrient cycling. Such quantification can help reveal the mechanisms by which animals control ecosystem dynamics as part of a concerted effort to enhance and revise the current working paradigm of biotic control over ecosystem functioning.

Disclosures

We have nothing to disclose.

Acknowledgements

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